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Electrolyte Equilibria in Blood

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ELECTROLYTE EQUILIBRIA IN BLOOD

By

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LIFE

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He was graduated from Lane Technical High School, Chicago, June, 1936; from Wright Junior College, Chicago, June, 1938; from the University of Illinois, June, 1940, with the degree of Bachelor of Science in Chemistry; and from Loyola University, June, 1944, with the degree of Master of Science.

From July, 1940 to March, 1947, he served as Technician, and as Assistant in Chemistry, under Dr. Julius Sendroy, Jr., in the Department of Experimental Medicine, at the Loyola University School of Medicine. He began his studies for the Doctor's degree in September, 1944, and in March, 1947, he was awarded the Reuben M. Strong Fellowship. He began the work for his dissertation in December, 1947.

The writer has published a paper with Julius Sendroy, Jr.: "Determination of carbon monoxide in gas mixtures," Journal of Biological Chemistry, 156 (1944) 61-75.

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Based on considerations formulated by Gibbs (16), Donnan (11) has shown that when a membrane separates two solutions of electrolytes, one of which contains one ion which cannot diffuse through the membrane, while all the other ions are diffusible, there will result an unequal distribution of the diffusible ions on the opposite sides of the membrane. At equilibrium the products of the concentrations of each pair of oppositely charged diffusible ions on the opposite sides of the membrane are equal. The manner in which Donnan arrived at these conclusions may best be described in his own words:

> "We suppose that the membrane (indicated in the following diagram by a vertical line) be impermeable for the anion R of a salt NaR (and also for the nondissociated part of the salt NaR), but permeable for all the other ions and salts to be considered in this connection. ..... Suppose that in the beginning we have a solution of NaR on one side of the membrane (indicated by a vertical line), and of NaCl on the other side

Na^+	Na^+
R^-	Cl^-
(1)	(2)

> In this case NaCl will diffuse from (2) to (1). In the end the following equilibrium will result:

Na^+	Na^+
R^-	
Cl^-	Cl^-
(1)	(2)

When this equilibrium is established, the energy required to transport reversibly and isothermally 1 gram molecule Na^+ from (2) to (1) equals the energy which can be gained by the corresponding reversible and isothermal transport of a gram molecule Cl^-. In other words, we consider the following infinitely small isothermal and reversible change of the system:

$$\partial n \text{ Mol } Na^+ \quad (2) \longrightarrow (1)$$

$$\partial n \text{ Mol } Cl^- \quad (2) \longrightarrow (1)$$

The energy which can be gained in this way (i.e., the diminution of free energy) is zero, hence:

$$(1) \qquad \partial n \cdot RT \log \frac{[Na^+]2}{[Na^+]1} + \partial n \cdot RT \log \frac{[Cl^-]2}{[Cl^-]1} = 0$$

or

$$(2) \qquad [Na^+]2 \cdot [Cl^-]2 = [Na^+]1 \cdot [Cl^-]1$$

where the brackets signify molar concentrations."

Wilson (58) has shown that the latter equation of Donnan can also be derived electrostatically. He points out that in passing from one phase to another, the oppositely charged ions must move in pairs, since they would otherwise set up powerful electrostatic forces which would prevent their free diffusion. The rate of transfer of the diffusible ions from one side of the membrane to the other would depend upon the frequency with which they chance to strike the membrane in pairs. This frequency is measured by the product of the concentrations of the ions in a given pair. At equilibrium, the rate of transfer of Na^+ and Cl^-, for example, from one side of the membrane to the other exactly equals the rate of transfer of these ions in the opposite direction, from which it follows that the product of the concentrations of these ions has the same value on both sides of the membrane. For

any number of mono-monovalent ionogens present in the system, the product of the concentrations of any pair of diffusible and oppositely charged ions will have the same value in both solutions.

Since on the side which contains the non-diffusible anion the concentration of cations Na is the sum of the cations in combination with the Cl plus the cations in combination with the non-diffusible anion, while on the other side of the membrane the concentration of the Na ions is only that of the Na in combination with Cl and equal to the concentration of the Cl, Donnan's equation can only be satisfied if

$$[Na^+]_1 > [Na^+]_2$$

and

$$[Cl^-]_1 < [Cl^-]_2$$

This difference in the concentration of the diffusible ions on opposite sides of the membrane leads to a potential difference between them which Donnan shows must, on the basis of Nernst's formula, be expressed by the following equation:

(3) $$\pi_1 - \pi_2 = \frac{RT}{F} \log \frac{[Na^+]_2}{[Na^+]_1} = RT \log \frac{[Cl^-]_1}{[Cl^-]_2}$$

Loeb (26) has tested this consequence of the Gibbs-Donnan theory for solutions of protein salts separated from water by a collodion membrane, with the result that the theory was completely confirmed.

Donnan's final equation can also be written as follows:

(4) $$\frac{[Cl^-]_1}{[Cl^-]_2} = \frac{[Na^+]_2}{[Na^+]_1}$$

It is this form which will be used throughout this paper, with the understanding that the equation applies to ion activities.

Barcroft, et al (5) in attempting to explain why the pH inside cells is lower than the pH of serum, suggested that a Donnan "membrane equilibrium" occurred at the boundary of the corpuscle. They were of the opinion that hemoglobin, because of its enormous molecular weight, could provide only a negligible part of the total ionic charge. Thus, they believed that any differences in distribution of H-ions (as well as the other diffusible ions) would be due to an inequality in the concentration of the cations (neglecting, for simplicity, the concentration of the non-diffusible phosphate anions, which they considered relatively small) on the two sides of the "corpuscular envelope." Since the basic ion concentration of the plasma was found by them to be practically constant, they believed that small natural differences and possibly small progressive changes, produced by time or by acclimatization, in the basic ion concentration inside the red corpuscle were the responsible factors.

Concurrently with the paper of Barcroft and his collaborators, Warburg (57) published a paper in which he discussed the unequal distribution of permeable ions across the red blood corpuscle membrane, and developed

equations in which the buffer effects of cell and plasma proteins were indicated. However, he was unable to calculate the actual amounts of base balanced by negative protein charges. At this time Van Slyke, Hastings, Heidelberger, and Neill (46) had derived an equation showing the amount of base bound by oxygenated hemoglobin and had found, because of its polyvalent acid character, it is able to combine with an appreciable amount of base. As used here, the word base refers to a substance which can gain a proton.

In 1923, by assuming that osmotic pressure is equal in cells and serum, and that the distribution of diffusible ions between cells and serum is influenced by the non-diffusible ions according to Donnan's law, Van Slyke, Wu, and McLean (55) were able to derive an equation showing "the approximate relationship between the distribution of diffusible ions and the amounts of alkali combined with the non-diffusible substances (proteins) of the cells and serum." Their equation took the form:

(5) $$\underline{r} = \frac{[H^+]_s}{[H^+]_c} = \frac{[Cl^-]_c}{[Cl^-]_s} = \frac{[BHCO_3]_c}{[BHCO_3]_s} =$$

$$1 - \frac{[BP]_c + [Hb]_c - [BP]_s}{2([B]_s - [BP]_s)}$$

where $\underline{r}$ is the distribution ratio, $[BP]_s$ represents the base bound to serum proteins, $[BP]_c$ the base bound to hemoglobin, and $[B]_s$ the total base of the serum. In order to apply this equation it was necessary for them to derive the equations expressing the amount of base bound by the proteins of the serum and cells. These equations were found by using the

techniques of Van Slyke, Hastings, Heidelberger, and Neill (46) to find the carbon dioxide-binding power, at various CO_2 tensions, of samples of serum and cells, which had been dialyzed and to which had been added controlled amounts of Cl and base, as HCO_3 (Na salts for serum, and K salts for cells). The pH of the solutions was derived from the Henderson-Hasselbalch equation $pH = pK' + \log \frac{[BHCO_3]}{[H_2CO_3]}$. On the basis of experimental data of Cullen (7), pK' was taken to be 6.12 for both serum and cells. By plotting the amount of base found to be bound by serum protein, from BP = Total base - bound CO_2, against pH a curve was obtained whose equation was $mM\,[BP]_s = 0.068\,[P]_s \cdot (pH_s - 4.80)$. In the case of cells two curves were obtained. The first, for reduced cells, was experimentally determined in the same manner as for serum. The second, for oxygenated cells, was obtained by correcting the $[BP]_c$ values obtained for reduced cells for the increase in equivalents of alkali bound by hemoglobin when 1 molecule of oxygen was added, and plotting these corrected values against pH_c. The equations for the two curves obtained are:

(6) for reduced blood, $[BP]_c = 3.35\,[Hb]_c \cdot (pH_c - 6.74)$ and

(7) for oxygenated blood, $[BP]_c = 3.6\,[Hb]_c \cdot (pH_c - 6.6)$

where $[Hb]_c$ is hemoglobin in mols per liter. From these two equations another could be derived for blood with varying degrees of oxygen saturation:

(8) $[BP]_c = 3.35\ [Hb]_c\ .(pH - 6.74) + [O_2]_c\ .(0.25\ pHc - 1.18)$

where $[O_2]_c$ is oxygen content in mols per liter. With these mathematical expressions developed by Van Slyke, Wu, and McLean, the distribution of electrolytes and water between cells and serum, and the manner in which the distribution is affected by changes in pH and oxygen content could be predicted. The effects of varying CO_2 tensions on the distributions ratios for Cl and HCO_3 in horse blood were investigated by Van Slyke, Wu, and McLean, and the results were found to approximate those predicted by their equation for r. Bound CO_2, $[HCO_3]$, present in serum and cells was determined by the equation

(9) $$[HCO_3] = [CO_2] - [H_2CO_3]$$

$[CO_2]$ is the total CO_2 content. $[H_2CO_3]$ represents the free, dissolved CO_2, and was determined by the equation:

(10) $$mM\ H_2CO_3 = \frac{\alpha\, pCO_2}{760 \times 0.0224}$$

α is Bunsen's solubility coefficient, representing the cc. of CO_2, measured at 0°, 760 mm., that are dissolved by 1 cc. of solution when under 760 mm. pressure of the gas. It was found by assuming that the proteins present in blood do not affect the solvent power of water, therefore, the solubility of CO_2 in serum and cells was thought to be the same as that for a 0.16 M salt solution. This had been estimated, from the data of Geffcken (15), to be equal to 98.8 per cent of the solubility in pure water. PCO_2, the CO_2 tension, was found by analyzing the gas phase with which the whole blood had been equilibrated. Serum and whole blood $[Cl]$ was determined directly

by the method of Austin and Van Slyke (4). Cell $[Cl]$ was calculated by difference. Van Slyke, Wu, and McLean believed that deviations between the Cl and HCO_3 ratios might be explained by variations in γ, the activity coefficient, in cells and in the serum, and that deviations of these two ratios from the calculated r might be due to the fact that the application of the gas laws to electrolyte solutions is approximate rather than exact.

In 1925, Van Slyke, Hastings, Murray, and Sendroy (48) re-emphasized the importance of the activity coefficient. An improved method (44) for determining chlorides, which allowed direct analysis of cells, was used, and as a result, the curve they obtained when the $[Cl]_c : [Cl]_s$ ratios were plotted against the pH of the serum at a given CO_2 tension was definitely lower than the $[HCO_3]_c : [HCO_3]_s$ curve. This was in agreement with the results found by Warburg (51) in 1922. The H^+ activities in reduced horse cells and serum were determined directly by the electrometric method, and the pK' of Hasselbalch's equation for cell contents was determined. A value of 5.93 was obtained. This pK' was then used in calculating the H^+ activities, by Hasselbalch's equation, in oxygenated blood, where the hydrogen electrode could not be used. The activities so estimated could be placed on a basis of measured electrometric H^+ activity values. From the relationship in the distribution ratios of H^+ activities and Cl and HCO_3 concentrations between serum and cells, the relative activity coefficients for chloride and bicarbonate in serum and cells were estimated. Their

experimentally determined distribution ratios were found to be related as follows:

$$(11) \qquad \frac{[\alpha H^+]_s}{[\alpha H^+]_c} = 0.77 \frac{[Cl]_c}{[Cl]_s} = 0.62 \frac{[HCO_3]_c}{[HCO_3]_s}$$

The distribution ratios for chloride, bicarbonate, and hydrogen were determined in reduced blood as well as in oxygenated blood in order to ascertain whether reduction, which results in a lesser amount of base being bound by hemoglobin (46,49), would cause the value of $\underline{r}$ to move nearer to 1, as could be predicted by the equation derived by Van Slyke, Wu, and McLean (55). This was found to occur. The changes observed in the distribution ratios with change in serum pH and in the degree of oxygenation of the hemoglobin approximated those predicted from changes in the base-binding power of cell and serum proteins caused by varying pH and oxygenation. This gave strong support to the validity of the equation derived by Van Slyke, Wu, and McLean (55) and to their assumption that the law of Donnan holds for the membranes of the blood cells. That the non-diffusible base bound by hemoglobin in the cells is the most improtant single constituent of blood in determining the distribution of chloride, bicarbonate, and hydrogen ions seems to be established by the above facts.

Hastings, Sendroy, McIntosh, and Van Slyke (21) made a study of the ionic distribution in blood freshly drawn from the human body. The analyses were all performed on the partially oxygenated venous blood. The $[Cl]_c : [Cl]_s$ and $[BHCO_3]_c : [BHCO_3]_s$ ratios were calculated using a correction

which gave the values that would be obtained had the blood been completely oxygenated. This correction was deduced from results obtained with oxygenated and reduced horse blood (48). The CO_2 tension of the blood was calculated from the total CO_2 content of the serum and the electrometrically determined pH_s by the following formula (3):

(12) $$p = \frac{[CO_2]}{0.0591 \alpha^{\circ} (10^{pH - pK'} + 1)}$$

The value of 6.10 for pK'_s found by Hastings, Sendroy, and Van Slyke (22) was used. The value of α° used, was that found by Van Slyke, Sendroy, Hastings, and Neill (49). $[H_2CO_3]_s$ was calculated using this new value for α°, and $[H_2CO_3]_c$ was calculated using the value of α° found for cells by these workers. The distribution ratios found for chloride and bicarbonate averaged several per cent higher, i.e., $\underline{r}$ was closer to 1, than in horse blood at the same pH_s. The difference approximates that which can be calculated by the equation of Van Slyke, Wu, and McLean (55) from the lesser base-binding power of the colloidal constituents of human cells. Comparison of the data of Van Slyke, Wu, and McLean (55) on horse cell contents with those of Adair (1) on human cell contents indicates that the colloids of the latter bind less base at a given pH. From these data it was calculated that the $\underline{r}_{Cl}$ and $\underline{r}_{HCO_3}$ should be about 0.09 higher in human than in horse blood at the same pH. Experimentally it was shown that this was approximately the case for both normal and pathological human blood. The fact that normal and pathological bloods gave similar results allowed these authors to conclude

"that the two factors which are theoretically most important in determining the values of the ratios at any given pH are the total base concentration in the serum and the hemoglobin concentration in the cells, and that neither of these is liable to great fluctuations in the conditions studied." The average of their determinations on human blood showed the distribution ratios to be related as follows:

$$\frac{[Cl]_c}{[Cl]_s} = 0.87 \frac{[BHCO_3]_c}{[BHCO_3]_s} \tag{13}$$

The difference in distributions was attributed to "factors which affect the activity coefficients of Cl and HCO_3 anions in the cells, and which we have thus far been unable to identify."

Harkins and Hastings (18) studied the distribution, *in vivo* and *in vitro*, of chloride and bicarbonate in dog blood in experimental acidosis. For the *in vivo* studies, five times the quantity of normal hydrochloric acid necessary to halve the blood bicarbonate *in vitro* was injected into a femoral vein of the dog. In general, when the chloride ratios were plotted against pH, the experimental points followed the curves plotted from the equation of Van Slyke, Wu, and McLean (55) for horse blood. In the case of the corresponding bicarbonate ratios there was fair consistency. They found the chloride ratio to be approximately equal to 1 at the isoelectric point of hemoglobin. This is in agreement with the equation of Van Slyke, Wu, and McLean. However, in the case of bicarbonate, the ratio exceeded unity long before the isoelectric point was reached. This suggested to them that the

calculated bicarbonate values for cells were much too high, due either to there being an appreciable amount of undissociated hemoglobin bicarbonate or to activity changes. In some of their experiments, where the pH was much below the isoelectric point, they found bicarbonate ratios much greater than unity (even allowing for the lowered activity in the cells). Since the Donnan distribution law predicts a higher concentration of diffusible anions in cells than in serum when the non-diffusible ion, in this case the hemoglobin acting as a base, is a cation, the authors came to the conclusion that the validity of the Van Slyke, Wu, and McLean hypothesis had been further strengthened.

The existence of a direct combination of CO_2 and hemoglobin was demonstrated by Bohr (6) who believed that this combination was one involving only that group of the hemoglobin molecule which also binds oxygen. Henriques (24) proposed that direct combination of CO_2 and $-NH_2$ occurs, similar to that demonstrated by Faurholt (12) with simple amino acids. Henriques, and also Margaria and Green (30), asserted that the CO_2 combined with a simple amino group of the oxygen linkage exclusively, and not with any other since they found that the maximum amount of CO_2 which combined directly with hemoglobin as carbamate was equal to the oxygen capacity. However, Stadie and O'Brien (40) showed that the CO_2 combined as carbamate may under suitable conditions be three or four times the oxygen capacity. They concluded that the carbamate combination is not limited exclusively to the oxygen-binding group, but involves many, if not all, of the $-NH_2$ groups of the hemoglobin molecule. They also showed that carbamate formation can

be demonstrated in the case of horse serum protein, confirming earlier work of Siegfried (39), thus furnishing further evidence that the combination is not limited to a prosthetic "gas" combining group. Stadie and O'Brien found that an initially alkaline solution of either oxyhemoglobin in or reduced hemoglobin at 38° showed 0 carbamate at isoelectric pH when brought to this pH by sufficient increase of the equilibrium p_{CO_2}. This was in line with the findings of Faurholt (12), Meldrum and Roughton (31), and Stadie and O'Brien (40), who showed, in the case of the simple amino acids, that the direct combination of CO_2 is with the amphanion ($COO^- \cdot R \cdot NH_2$) only, and not with the zwitter ion ($COO^- \cdot R \cdot NH_3^+$). Estimations of carbamino-CO_2 in oxygenated and reduced cell hemolysate have been made by Ferguson and Roughton (14) and by Ferguson (13). Stadie and O'Brien (41) were able to prove that one equivalent of hydrogen ions is ionized for every mole of CO_2 bound as carbamate. Accordingly, the equation of the reaction, in the case of hemoglobin, is

(14) $$Hb^- + CO_2 = HbAm^- + H^+$$

where $HbAm^-$ represents carbamate. By substituting $\alpha_{CO_2} \cdot PCO_2$ for CO_2, and applying the law of mass action they obtained the equation

(15) $$K_{Am} = \frac{(H^+)\ (HbAm^-)}{\alpha_{CO_2} \cdot PCO_2\ (Hb^-)}$$

By substituting $(HCO_3^-)\ (H^+)\ /K'_{CO_2}$ for $\alpha_{CO_2} \cdot P_{CO_2}$ (since $K'_{CO_2} = \frac{(HCO^-)\ (H^+)}{\alpha_{CO_2} \cdot pCO_2}$ and then substituting $(\overline{CO_2})$ - $(HbAm^-)$ for (HCO_3^-) (since

$(\overline{CO_2}) = (HCO_3^-) + (HbAm^-)$, where $(\overline{CO_2})$ represents total bound CO_2) they obtained the following equation for determining the proportion of total bound CO_2 existing as carbamate

(16) $$\frac{(HbAm^-)}{(\overline{CO_2})} = \frac{1}{1 + \frac{1/K_{Am}}{\frac{K'_{CO_2}}{(Hb^-)}}}$$

By this equation, and one for proteins in general, where Pr^- replaces Hb^- and $PrAm^-$ replaces $HbAm^-$, they could calculate the partition of the total CO_2 as bicarbonate and carbamate in the cells and in the plasma of whole horse blood.

Dill, Daly, and Forbes (8) redetermined the pK' values for human serum and reduced and oxygenated cells at 37°, "the temperature of man," using the solubility coefficients for CO_2 found by Van Slyke, Sendroy, Hastings, and Neill (53). In agreement with Hastings, Sendroy, and Van Slyke (22), they found a pK' for serum of 6.11. In the usual physiological pH range of human blood the pK' for cells in the oxygenated state was found to be 6.04, and for cells in the reduced state it was found to be 5.98. This latter value is only 0.01 higher than the recalculated value found by Van Slyke, Hastings, Murray, and Sendroy (48).

Dill, Edwards, and Consolazio (9) made a study of the distribution ratio, $\underline{r}$, for combined CO_2, chloride, and the hydrogen ion in relation to pH_s of oxygenated and reduced blood from man at rest. Their curve for $\underline{r}_{HCO_3}$ plotted against pH_s of oxygenated blood was found to fit very closely the values found earlier by Dill, Daly, and Forbes (8). There was also agreement

with the work of Hastings, Sendroy, McIntosh, and Van Slyke (21) with oxygenated blood. However, Dill, Edwards, and Consolazio found a wide divergence of the curves for oxygenated and reduced blood in the alkaline range, a characteristic which they thought was not in accord with the theory proposed by Van Slyke, Wu, and McLean (55). The curves derived for $\underline{r}_{Cl}$ were in better accord with the theory, and the values obtained for oxygenated blood were in agreement with those found by Hastings, Sendroy, McIntosh, and Van Slyke (21). The pH of oxygenated and reduced red cells was calculated by use of the Henderson-Hasselbalch equation with the values of pK' given by Dill, Daly, and Forbes (8), and curves for the distribution of the hydrogen ion in relation to pH_s were derived. They found that $\underline{r}_H$ is unique in that the value for reduced blood is less than that for oxygenated blood in the alkaline range, i.e., the curves for oxygenated and reduced blood intersect.

Dill, Talbot, and Consolazio (10) found that the distribution of combined CO_2 in blood of man is not appreciably changed at high altitudes, but that there is probably a slight reduction in $\underline{r}_{Cl}$.

Dill, Edwards, and Consolazio (9) used the values they obtained for oxygenated and reduced blood to convert values which they found for arterial blood into synthetic venous blood values. They then determined $\underline{r}_{HCO_3}$ and $\underline{r}_{Cl}$ for arterial and venous blood. By applying Ferguson's (13) estimations they calculated the $HbCO_2$ content of arterial cells and of venous cells. These values were used to calculate the distribution of the other forms of combined CO_2 between cells and serum. The distribution ratio in arterial blood for combined CO_2 other than $HbCO_2$ was found to be the same as the distribution

ratio for chloride. However, in venous blood the ratio proved to be much less than the chloride ratio. These authors suggest that Ferguson's measurements, which were done on homogenous hemolysate, may not be applicable to whole blood where there is an opportunity for dividing the transport of CO_2 between cells and serum.

Dill, Edwards, and Consolazio developed equations for calculating BP_c, the base bound by hemoglobin and other non-diffusible constituents in human red cells. It was assumed that the pH_c of minimal base-binding is the same for human hemoglobin as that given by Van Slyke, Wu, and McLean (55) for horse hemoglobin. They corrected these pH_c values for the revised pK'_c of Dill, Daly, and Forbes (8). Titration curves were plotted in a manner similar to that of Van Slyke, Wu, and McLean, and the following equations were derived:

For oxygenated blood

(17) $$BP_c = HbO_2 \cdot [-0.5(pH_c)^2 + 10.625pH_c - 48.46]$$

For reduced blood

(18) $$BP_c = Hb \cdot [-0.214(pH_c)^2 + 6.207pH_c - 31.97]$$

In these equations, BP_c is expressed in milli-equivalents per liter. The concentration of oxyhemoglobin, HbO_2, is expressed in terms of mM of combined oxygen per liter of blood. The millimolar concentration of reduced hemoglobin, Hb, is the difference between the oxygen-combining capacity and the combined oxygen present.

Rapoport and Guest (35), using the corrections required for carbamate as indicated by Meldrum and Roughton (31), Ferguson and Roughton (14), Ferguson (13), and Stadie and O'Brien (40), and the pK'_c found by Dill, Daly, and Forbes (8), recalculated the data of Van Slyke, Hastings, Murray, and Sendroy (48), and found the distribution ratios for bicarbonate, chloride, and hydrogen ion to agree closely. The relationships were

for reduced blood:

(19) $[H^+]_s/[H^+]_c = 0.91\,[Cl]_c/[Cl]_s = 0.94\,[HCO_3]_c/[HCO_3]_s$.

for oxygenated blood:

(20) $[H^+]_s/[H^+]_c = 1.06\,[Cl]_c/[Cl]_s = [HCO_3]_c/[HCO_3]_s$.

However, upon recalculation of the pH of the cells, with the pK'_c determined by Dill, Daly, and Forbes, and the value for the solubility coefficient of CO_2 determined by Van Slyke, Sendroy, Hastings, and Neill, they show the hydrogen ion distribution ratios found by Van Slyke, Wu, and McLean, to be lowered. They also point out that recalculation of the theoretical distribution ratios using a molecular weight of 66,800 for hemoglobin, based on data of Adair (2) and Svedberg and Nichols (43), instead of the 16,700 used by Van Slyke, Wu, and McLean, leads to a higher value of $\underline{r}$. Rapoport and Guest suggest that concentration and anion equivalency of hemoglobin and also of diphosphoglycerate should be taken into account in using the equation of Van Slyke, Wu, and McLean to calculate the theoretical distribution ratios of diffusible ions. When this was done, it demonstrated fair agreement

between theoretical ratios and observed distribution ratios for chloride, bicarbonate (corrected for carbamate CO_2 in the cells), and hydrogen ions in dog blood, before and after pyloric obstruction. Thus, support for the validity of the Donnan theory as applied to dog blood was offered.

Peters, Tulin, Danowski, and Hald (34) made an analysis of data, collected in their laboratory over a period of twenty years (33, 34, 56), dealing with the effects of varying CO_2 tension; of adding chloride or bicarbonate salts of sodium and potassium, water and isotonic sucrose solutions; and of autoglycolysis, on the distributions of combined CO_2 and chloride between cells and plasma of oxygenated blood. The response of the distribution coefficient for bicarbonate to a given change of pH was found to be greater when this is produced by altering bicarbonate than it is when CO_2 tension is varied. They concluded from this that the variation of distribution coefficients with pH seems to depend upon the manner in which pH is altered. They found that the addition of chloride affected the distribution coefficient for chloride more than the distribution coefficient for bicarbonate, whereas the addition of bicarbonate has a greater effect on the distribution coefficient for bicarbonate. They concluded from this that when a salt of either chloride or bicarbonate is added to blood, the distribution coefficient of the added component rises more rapidly than that of the other component because the increment contributes more to the active than to the inactive fraction. They found no real redistribution of chloride or bicarbonate when isotonic sucrose solution was added in amount sufficient to approximately double the volume of the serum. This led them to state: "If

blood is regarded as a system in which two media are separated by a membrane impermeable to cations and a large proportion of anions, but freely permeable to water, it is clear that the addition to blood of an isomolar solution of a nonpenetrating nonelectrolyte should have no other effect than to dilute in the serum those electrolytic components which are not restrained. On the other hand, the Gibbs-Donnan principle would require that the anions and cations to which the membrane is permeable should redistribute themselves according to a definite rule which, in blood, appears to be violated."

In view of the apparent discrepancies which have been indicated, a thorough re-investigation of the distribution of the electrolytes between cells and plasma, within the ranges of normal physiological conditions as well as under certain abnormal pathological conditions, seems desirable. Therefore, experiments have been carried out designed to test further the validity of the application of Donnan's law to the red blood cell membrane, and to explain existing variations from expected theoretical values.

The distribution coefficients for chloride, bicarbonate, and hydrogen ions in normal human blood have been determined. A study has been made of the effect of oxygenation of the hemoglobin, and of alteration of the pH of the blood by varying the CO_2 tension. The work of Peters' group (34), in which the effect on the distribution ratios for chloride and bicarbonate of an increase in chloride or bicarbonate concentration was studied, has been repeated, and the effect of lowered chloride or bicarbonate concentration has

also been studied. A study of the distribution ratio for the hydrogen ion has been included in these experiments. In view of the fact that Peters' group studied only the redistribution of chloride and bicarbonate after the addition of isotonic sucrose solution to blood, it seemed desirable to study the effect that this addition has on the distribution ratios for chloride, bicarbonate, and hydrogen ions. Therefore, experiments have been carried out which determine this effect.

Factors which seemed likely to have a bearing upon the distribution coefficients have been carefully controlled, and certain refinements of previous techniques have been developed. The termperature of the blood has been held constant throughout all the manipulations to which the blood was subjected, until the cells and serum were separated. Because Harris (19) has stated that there is a metabolic function, related to glycolysis, which is responsible for the manner in which the cations, sodium and potassium, are distributed across the human red blood cell membrane, the effect of glycolysis upon the distribution of diffusible ions has been studied and controlled. A new technique has been developed which permits separation of cells and serum, or plasma, without exposure to air, so that loss of CO_2 has been prevented completely. Electrometric measurements of the hydrogen ion activity in the cells and serum of both oxygenated and reduced blood have been made, by the use of a glass electrode.

Procedure

Initial treatment of blood - Approximately 100 ml. of human venous blood, drawn mostly from medical students, was collected in an Erlenmeyer flask and defibrinated by whipping with a glass rod, without delay. The defibrinated blood was usually filtered through gauze. After this treatment, the blood was found to have a serum pH of approximately 7.4 and an oxygen-saturation of approximately 75 per cent. The per cent of cells (cell volume) present was determined by centrifuging samples in Wintrobe hematocrit tubes at 2,500 r.p.m. Readings were taken at 15 minute intervals. Usually four such periods of centrifugation were required to attain constant volume. The amount of hemoglobin present in the whole blood was determined by the Sendroy oxygen capacity method (36). The solubility factor established by Sendroy, Dillon, and Van Slyke (37) was used in making corrections for physically dissolved O_2.

Attainment of desired CO_2 and O_2 tensions - The blood was poured into two specially constructed centrifuge tubes (Fig. 1), with capacities of 45 ml., containing an outlet and a capillary tube leading to the bottom. The capillary tube, A, was filled with Hg and closed at the top with a piece of rubber tubing and a small clamp. The delivery tube, B, was then connected, by means of approximately 7 cm. of rubber tubing, to a 300 cc. tonometer containing a desired gas mixture. The blood was equilibrated with the

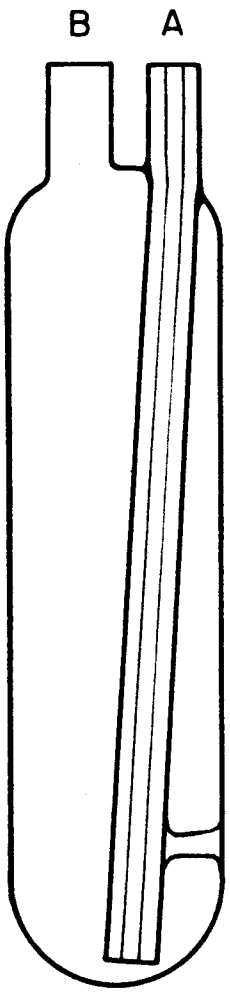

Fig. 1. Modified centrifuge tube for separation of serum and cells.

gas mixtures in a manner similar to the first saturation method of Austin, Cullen, Hastings, McLean, Peters, and Van Slyke (4). The equilibrations were carried out at atmospheric pressure, and the final CO_2 tension was determined by analysis. The tonometer and centrifuge tube were then submerged in a water bath, which was maintained at 37° C. $\pm$ 0.01° and allowed to attain approximately this temperature. Atmospheric pressure was again established by opening the stopcock of the tonometer. All of the liquid phase in the centrifuge tube was allowed to enter the tonometer and the containers were rotated in a lateral position in the bath for fifteen minutes. The liquid phase was allowed to flow back into the centrifuge tube and atmospheric pressure was established by opening the tonometer stopcock. The liquid phase was returned to the tonometer and the containers rotated for fifteen minutes more. The liquid phase was returned to the centrifuge tube once more and the connecting rubber tube was closed by a clamp. A new gas mixture, identical to the previous one, was prepared in the tonometer and the two fifteen minute saturations as described above were repeated. This overall saturation of one hour was found sufficient to establish equilibrium in these systems. The blood was allowed to flow back into the centrifuge tube as completely as possible. The connecting tube was closed with a small clamp near the outlet of the centrifuge tube and with another clamp near the opening of the tonometer. The rubber tubing was cut. The 300 cc. tonometer was set aside for analyses of the gas phase.

The centrifuge tube was submerged in a small water bath maintained at

37° C. The small portion of the gas remaining with the blood was removed by displacement with Hg run through the capillary tube. The two clamps were then tightened.

Separation of serum and cells - The blood was then centrifuged for thirty minutes at approximately 2,200 r.p.m. at a temperature of 37° C. 0.5° maintained within the centrifuge drum by means of a heater and a thermoregulator. The centrifuge tubes were again placed in the small 37° C. water bath and the serum and cells were separated by transfering them to small vessels, over Hg, by displacement as shown in Fig. 2. The first 1 to 2 cc. of serum was wasted, after which serum and cells, unexposed to air, were collected. Two vessels were used for holding the cells of each portion of blood. To the first vessel, containing 0.15 cc. of 5 per cent saponin, there were delivered 5 ml. of cells to be hemolyzed for pH determination; the second vessel, without saponin, received the remainder of the cells to be used for CO_2, Cl, and H_2O determinations. The serum obtained by this method was almost completely free of cells, while the cells contained 3 per cent of serum by volume as determined by high speed centrifugations to constant volume of samples in 10 ml. "milk-fat" centrifuge tubes.

The pH and CO_2 content of the cells, and the CO_2 tension of the gas phase were determined as soon as possible to minimize errors due to further glycolysis. The remainder of the necessary determinations were done when convenient.

Determination of pH of cells and serum - The 12 ml. vessel, containing

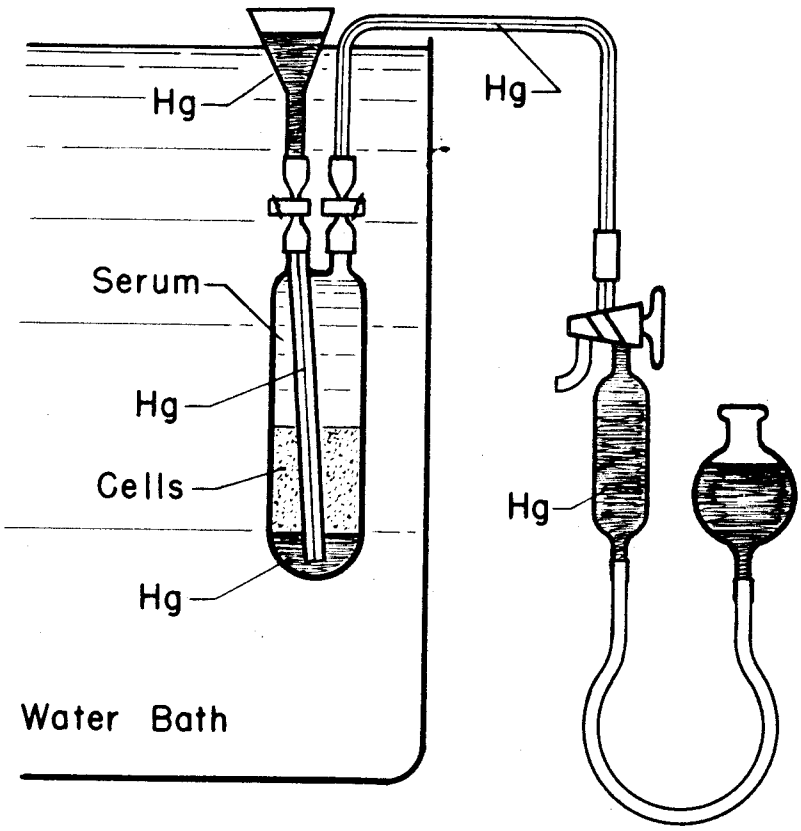

Fig. 2. Apparatus used for separating serum and cells.

the hemolyzed cells, and its Hg leveling bulb were placed in an incubator which housed the glass electrode, and which was maintained at 37° C. The glass electrode system consisted of a micro glass electrode of the type described by MacInnes and Belcher (27), in conjunction with a Thermionic Amplifier, used with a suitable galvanometer to attain a sensitivity of ± 0.003 pH, with an accuracy of -0.011 pH (38). When the vessel and its contents attained the temperature of the incubator, the cells were transferred to the glass electrode through a piece of glass capillary tubing by Hg displacement. After wasting the first 1 to 2 ml., this method permitted transfer without exposure to air. The pH was measured on several successive portions, each of which displaced the previous one. After the pH of each sample of cells was measured, the glass electrode was washed thoroughly with isotonic NaCl solution and then with distilled water. In spite of this procedure, there seemed to be a gradual layering of protein on the glass membrane which, in time, caused a drifting of the e.m.f. being measured. This was eliminated by cleaning the glass electrode with chromic acid. Extreme precautions were required so that localized heating, due to the contact of chromic acid with water, did not crack the membrane. The pH of the cells was again measured after approximately four hours had elapsed from the time at which the cells and serum had been separated. The pH of the serum was measured in the same manner as the cells. $[\alpha H^+]$ was calculated from pH.

A 0.05 M potassium acid phthalate solution was used as a standard (28).

This in turn was standardized against a phosphate buffer solution of known pH (according to Hastings and Sendroy (20), containing 20 parts M/15 KH_2PO_4 to 80 parts M/15 Na_2HPO_4.

Determination of CO_2 content of serum and cells - The determination of the CO_2 content of the serum was done according to Van Slyke and Neill (50). However, weighed samples were used so conditions involving CO_2 loss simulated those for cell analyses, for which measurement of samples by volume is difficult. The serum, and cells, were forced up into a one ml. stopcock pipette so that there was no air space left in the pipette. A cap was placed over the opening while the samples were being weighed, and was removed when the samples were introduced into the reaction chamber of the Van Slyke -Neill manometric blood gas apparatus. Approximately three minutes elapsed from the time the sample was removed from the vessel and transferred to the reaction chamber. Control experiments were carried out in which samples of serum were measured by volume, and the amount of CO_2 found per gram (from specific gravity determinations) was compared to that found in weighed samples. Of the CO_2 present in serum, approximately 0.3 per cent is lost in the time required for weighing the samples. Since this loss is so small, it has been assumed that approximately the same amount of CO_2 present in the cells could be lost in this time. Consequently these losses would cancel one another when ratios for cells to serum are calculated. Two and one-half ml. of 0.1 N lactic acid were used to free the CO_2 from the serum. Six ml. of N/60 lactic acid were used to free the CO_2 from the cells (21). An

extraction period of six minutes was required. The pressure of the extracted CO_2, p_1, was measured at 2.0 cc. volume. The CO_2 from serum was absorbed with 0.3 ml. of 5 N NaOH. For cells, 1 ml. of air free 1 N NaOH was used. p_2 was measured at 2.0 cc. Blank analyses were carried out, with the appropriate lactic acid solution replacing the serum or cells.

Determination of CO_2 in the gas phase - Two methods were used for determining the CO_2 tension of the gas phase with which the blood had been equilibrated, depending upon the amount of CO_2 used. For the experiments in which 40 mm. and 100 mm. of the CO_2 were used, the Van Slyke and Sendroy absorption method (52) was used. A sample of the gas was measured in the Van Slyke-Neill apparatus, and the diminution of pressure noted after absorption of the CO_2 in the chamber with sodium hydroxide was used to calculate the concentration of the CO_2 in the gas. When only 15 mm. of CO_2 was used, the Van Slyke, Sendroy, and Liu isolation method (54) was used. The CO_2 in a sample of the gas was first isolated from the other gases by absorption with alkali solution in the chamber of the Van Slyke-Neill apparatus. The other gases were then ejected, the absorbed CO_2 was set free by acid, and was determined as in serum analyses.

Determination of chloride in serum and cells - Inasmuch as a digestion method provides the best available procedure for direct analysis of chloride present in cells, as a matter of uniformity and convenience, the Sunderman-Williams method (42), with slight modifications, was used for all chloride analyses. One ml. samples of serum and cells were weighed in stoppered

125 ml. Erlenmeyer flasks. Fifteen ml. of 1 N KOH were added and the contents of the flasks were digested on a steam bath for one and one-half hours, being swirled occasionally, in order to saponify any fats present. Blank analyses, containing only the 15 ml. of KOH, were treated in the same manner. After cooling to room temperature, two drops of methyl orange indicator were added to each flask. Six N HNO_3 was added to the blank, drop by drop, with gentle agitation until the end point of the indicator had been reached, and the amount required was noted. The same quantity of HNO_3 was then added to the flasks containing the serum and cells. The end point for the indicator could be seen in the serum solutions but could not be seen in the cell solutions. However, a coagulation of the cell protein gave a visible indication that the end point had been reached. One ml. of 0.15 N $AgNO_3$ was then added to each of the flasks[1], followed by 5 ml. of concentrated HNO_3. The contents of the flasks were digested on a steam bath overnight. This caused the black sediment in the cell suspensions to disappear leaving a clear yellow solution. The solutions were cooled to room temperature and the sides of the vessels washed with a small amount of distilled water. One ml. of a saturated solution of ferric alum was added, and the solutions were titrated with 0.02 N NH_4CNS until the first definite pink coloration persisted after shaking. The NH_4CNS solution was standardized by titration of weighed amounts of fused $AgNO_3$ dissolved in approximately 20 ml. of water

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1 In experiments in which NaCl was added to the blood, 1.50 ml. of 0.15 N $AgNO_3$ was added.

to which was added 5 ml. of concentrated HNO_3 and 1 ml. of the saturated ferric alum solution.

Determination of H_2O present in serum and cells - The per cent of water present in serum and cells was determined by weighing 1 ml. samples in weighing bottles containing two pieces of 9 cm. filter paper, previously dried for two hours at 110° C., and drying the contents overnight at 110° C.

Determination of per cent oxygen-saturation of whole blood - The per cent oxygen-saturation of the equilibrated blood (corrected for dissolved O_2 with the factor of Sendroy, Dillon, and Van Slyke (37) was determined as described by Van Slyke and Neill (50). Because the removal of samples of the whole blood from the modified centrifuge tube before centrifugation was not deemed advisable, these determinations were made on another portion of blood, equilibrated with gas mixtures similar to those being used for equilibration with the blood in the modified centrifuge tubes. This indirect method was used after it had been established that the value obtained was comparable to the value found by direct analysis of the blood in the modified centrifuge tube within limits which resulted in negligible errors in the carbamate calculations.

Control of cell pH measurements - Although control experiments showed that when blood was one day old the CO_2 content did not change appreciably within a period of several hours, it was found that the pH of the cells did change slightly, for the most part, presumably due to the formation of non-volatile acids. Therefore, the change in pH with time was determined for

each sample of cells. By extrapolation, the pH at "zero time", the time after which slight changes occurring in the cells are not completely transferred to the serum, was determined. This time was arbitrarily set at 15 minutes after centrifugation had started. Because the change in pH with time was small, of the order of 0.015 pH units per hour, and fairly uniform, an error in estimating the "zero time" is negligible.

The change in pH of cells from fresh blood with time was found to be of the order of 0.040 pH units per hour, and this rate of change varied, being less as time progressed. Since the exact "zero time" was not known (this time would probably vary, depending on the level of the cells), it was not possible to get an accurate estimation of the pH of the cells in equilibrium with the serum by the method of extrapolation used for one day old blood. The addition of NaF to the cells decreased the rate of change in pH. However, the NaF could only be added after separation of the serum, since the purpose of this series of experiments was to study electrolyte equilibria while glycolysis was still active. The rate of change of pH would, therefore, be greater during the latter part of centrifugation and the time required for removing the cells from the serum, i.e., before the NaF is added. As a result, a measure of the change in pH with time after NaF was added is inadequate. The relative increase of CO_2 in the cells during the time required for centrifugation and the removal of the cells was found to be negligible, and further formation of CO_2 was stopped almost completely by the addition of NaF to the cells. Enough NaF was placed in the vessel

receiving the cells for CO_2 analyses to form a 0.25 per cent solution. Nothing was added to the cells being used for chloride and water determinations.

Calculations

The pH of serum or cells is calculated as

(21) $$pH = \frac{E - e}{\frac{2.3026RT}{F}}$$

in which E is the total e.m.f. of the system, e is the resultant e.m.f. of the silver-silver chloride and the mercury-calomel electrodes, R is the gas constant in volt-coulombs per degree, F is Faraday's constant in coulombs, and T is the absolute temperature. The value of e is obtained by standardization with 0.05 M potassium acid phthalate, the pH of which is taken to be 4.023 at 37.0° C. (29). Corrections are applied to the pH values calculated in this manner, based on determinations with a phosphate buffer having a pH value of 7.366 at 37.0° C. (20). (See Table I.).

$[\alpha_{H^+}]$ is calculated as

(22) $$[\alpha_{H^+}] = \text{-antilog pH}$$

The total CO_2 content of serum and cells in millimols per kilo of sample is calculated as

(23) $$\text{mM } CO_2/\text{kgm.} = pCO_2 \times f \times \frac{G}{W} \times \frac{1}{G}$$

in which f is the revised factor of Van Slyke and Sendroy (51) for 1.0 ml. samples at 2.0 cc. volume, and with S equal to 3.5 ml. for serum and 7.0 ml. for cells. G is the specific gravity of the serum or cells, and W is the weight of the sample. The ratio $\frac{G}{W}$ corrects f for weighed samples, and

TABLE I

Correction Factor for 0.05 Potassium Acid Phthalate Buffer

Standard: 20 parts M/15 KH_2PO_4:80 parts M/15 Na_2HPO_4

Exp. No.	Calculated pH_{37^o}	Observed pH_{37^o}	Correction
1.	7.366	7.355	0.011
2.	7.366	7.340	0.026
3.	7.366	7.353	0.013
4.	7.366	7.344	0.022
5.	7.366	7.344	0.022
6.	7.366	7.348	0.016
		Amount to be added to measured pH:	0.018

$\frac{1}{G}$ converts the calculated value from millimols per liter to millimols per kilo. pCO_2, the pressure of carbon dioxide at 2.0 cc. volume, is calculated as

(24) $$pCO_2 = p_1 - p_2 - c$$

p_1 and p_2 are discussed above (p.27) and c is obtained from readings in the blank analyses.

The value for cells is corrected for the presence of 3 per cent of serum by the equation

(25) $$\text{mM } CO_2/\text{kgm. cells (corr.)} = \frac{100 \times \text{mM } CO_2/\text{kgm. cells} - 3 \times \text{mM } CO_2/\text{kgm. serum}}{97}$$

The values for cells from fresh blood were increased by three per cent, to correct for the addition of saponin solution.

Millimols of CO_2 per kilo of water is calculated by the equations:

(26) for serum, $$\text{Total } [CO_2]_s = \text{mM } CO_2/\text{kgm.} \times \frac{100}{H_2O_s}$$

(27) for cells, $$\text{Total } [CO_2]_c = \text{mM } CO_2/\text{kgm.} \times \frac{100}{H_2O_c}$$

H_2O_s is the per cent of water in serum, and H_2O_c is the per cent of water in cells.

The CO_2 tension, in mm. Hg, is calculated as

(28) $$pCO_2 = \frac{\text{per cent } CO_2 \times (B - W)}{100}$$

Per cent CO_2 is determined by analysis of the gas sample; B is the barometric pressure, and W is equal to 47.2, the vapor pressure of water at

37.0° C.

The amount of physically dissolved CO_2, in millimols per kilo of water, is calculated by the equations:

(29) for serum, $[H_2CO_3]s = \frac{1000 \times \alpha \times pCO_2}{760 \times 22.24 \times G_s} \times \frac{100}{H_2O_s}$

(30) for cells, $[H_2CO_3]c = \frac{1000 \times \alpha \times pCO_2}{760 \times 22.24 \times G_c} \times \frac{100}{H_2O_c}$

α is the cc. of CO_2, measured under standard conditions, dissolved per cc. of fluid under 760 mm. CO_2 pressure. For serum, α is taken as 0.522 at 37.0° C. For cells, α is taken as 0.450 at 37.0° C. These values were derived from the values at 38.0° C. given by Van Slyke, Sendroy, Hastings, and Neill (53), by assuming the temperature effect is the same as on αCO_2 in water. G_s is the specific gravity of the serum, determined by weighing 1.0 ml. samples. G_c is the specific gravity of cell suspensions, assumed in all cases to be equal to 1.106.

The total bound CO_2, in millimols per kilo of water, is calculated as

(31) for serum, $[\overline{CO_2}]s$ = Total $[CO_2]s$ - $[H_2CO_3]s$

(32) for cells, $[\overline{CO_2}]c$ = Total $[CO_2]c$ - $[H_2CO_3]c$

The bicarbonate concentration in millimols per kilo of water, is calculated as

(33) for serum, $[HCO_3]s$ = $[\overline{CO_2}]s$ - serum carbamate

(34) for cells, $[HCO_3]c$ = $[\overline{CO_2}]c$ - cell carbamate

Carbamate is calculated by the equations of Stadie and O'Brien (41):

(35) for serum, $$[Pr_{Am}^-] = \frac{1}{1 \frac{1/K_{Am}}{K'CO_2}} \times [\overline{CO_2}]_s$$
$$(Pr^-)$$

(36) for cells, $$[Hb_{Am}^-] = \frac{1}{1 \frac{1/K_{Am}}{K'CO_2}} \times [\overline{CO_2}]_c$$
$$(Hb^-)$$

The value of $K_{Am}/K'CO_2$ for serum at 37° C. is taken as 2.2. The value of $K_{Am}/K'CO_2$ for completely oxygenated cells at 37° C. is taken as 3.2, and for completely reduced cells at 37° C. as 8.2. These values were derived from the values at 38° C. given by Stadie and O'Brien (41). The change in K_{Am} due to a change in temperature from 38° C. to 37° C. is calculated by using the van't Hoff equation

(37) $$2.3 \log \frac{K}{K'} = \frac{Q}{R}\left(\frac{1}{T} - \frac{1}{T'}\right)$$

Q is taken to be 17,000 calories as found by Stadie and O'Brien (41). A 5 per cent decrease in K_{Am} is found. K'_{CO_2} decreases by one per cent in going from 38° C. to 37° C. This is derived from the knowledge that pK'_{CO_2} increases by 0.005 units for a temperature decrease of 1° C. (8). The assumptions made by these authors, that the values of $K_{Am}/K'CO_2$ for human blood are the same as for horse blood, and that the values vary proportionately with the degree of oxygenation of the hemoglobin, have also been assumed in this paper. (Pr^-) is calculated by equation (12) of Van Slyke, Hastings, Hiller, and Sendroy (47):

(38) $(Pr^-) = BP_s = 0.104 \times \text{grams of protein} \times (pH_s - 5.08)$

The value, 70 grams of protein per liter, is used for all sera for which specific gravity determinations indicate that this value is within a range which permits adequate accuracy. In the sucrose experiments half of this value is used. (Hb^-) is calculated by the equations of Dill, Edwards, and Consolazio (9):

(39) for oxygenated blood,

$$(Hb^-) = HbO_2 \cdot [-0.5(pH_c)^2 + 10.625pH_c - 48.46]$$

(40) for reduced blood

$$(Hb^-) = Hb \cdot [-0.214(pH_c)^2 + 6.207pH - 31.97]$$

HbO_2 represents the combined oxygen present, and is expressed in terms of mM per liter of blood. It is obtained by multiplying the oxygen-combining capacity of the blood by the per cent saturation of hemoglobin found for each group of equilibrium gas mixtures (see Table II). Hb is found by subtracting HbO_2 from the oxygen-combining capacity of the blood. Hematocrit values are used to convert milli-equivalents per liter of blood to milli-equivalents per liter of cells.

The concentration of chloride, in millimols per kilo of sample, present in serum and cells is calculated in the usual stoichiometric manner. The value for cell chloride is corrected for the presence of 3 per cent of serum as shown for CO_2 in equation (23). Millimols of chloride per kilo of water in serum and cells is calculated as shown for CO_2 in equations (24) and (25).

TABLE II

Per Cent Oxygen Saturation of Hemoglobin in Reduced and Oxygenated Blood

	CO_2 Tension mm. Hg	O_2 Capacity mM per l.	O_2 Content mM per l.	Per Cent Saturation
Reduced Blood	15	9.00	0.366	4.1
"	40	8.56	0.225	2.6
"	100	9.00	0.509	5.6
Oxygenated Blood	15	9.62	9.48	98.6
"	40	9.62	9.47	98.5
"	100	8.82	8.56	96.7

The per cent of water in serum and cells is calculated from the loss in weight of the samples. The value for cells is corrected for the presence of serum as shown for CO_2 in equation (23).

Experimental

Experiments were carried out in which blood was equilibrated with gas mixtures containing approximately 15, 40, or 100 mm. of CO_2. Studies were made on oxygenated blood, by using CO_2 and air, and on reduced blood, by using CO_2 and H_2. Usually experiments with oxygenated and reduced blood at the same CO_2 tension were carried out simultaneously, on separate portions of the same blood. In the first series of experiments, the results of which are recorded in Tables III and IV, the blood was allowed to stand overnight, that glycolysis would be at a minimum on the day of the experiment (25). This was done in order to eliminate as much as possible the change in pH and CO_2 content of the cells after separation from the serum.[2] A second series of experiments were performed in which fresh blood was used. No attempt was made to interfere with or decrease the normal glycolysis. Within an hour after the blood was collected, it was equilibrated with an air, or H_2, mixture containing CO_2 at a desired tension. The results of these experiments are recorded in Tables V and VI. For the third series of experiments, the

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2 Because the addition of salts was to be avoided in this series of experiments, a glycolysis inhibitor was not added to the blood. Instead, glycolysis was allowed to continue for twenty four hours after the blood was drawn. As a result of this action, lactic acid is formed. Because of this, there is a decrease in the amount of available base, so that the CO_2- combining power and pH of the one day old blood are lower than they are for fresh blood.

TABLE III

RESULTS OF ANALYSES OF 1 DAY OLD BLOOD EQUILIBRATED WITH CO_2 AND AIR AT 37° C.

EXP. NO.	p_{CO_2}	Hemoglobin	Cell Volume	Cell H_2O	Serum H_2O	Cell Carbamate	Serum Carbamate	$[H_2CO_3]_c$	$[H_2CO_3]_s$
	mm.Hg	mM.per l. blood	per cent	per cent	per cent	per cent	per cent	mM./kilo H_2O	mM./kilo H_2O
					15 mm. CO_2				
1.	21.6	9.65	49.3	64.7	91.0	12.18	3.61	0.80	0.72
2.	17.2	8.61	45.8	65.1	91.4	11.42	3.61	0.64	0.57
3.	17.5	8.93	45.9	64.2	92.0	12.90	3.70	0.66	0.57
4.	16.0	8.72	46.5	64.6	91.3	11.83	3.65	0.60	0.53
					40 mm. CO_2				
5.	43.5	9.62	50.4	63.6	91.0	10.35	3.34	1.65	1.45
6.	42.6	8.62	49.4	66.0	90.8	8.93	3.32	1.55	1.42
7.	45.0	8.76	46.6	65.7	91.1	9.80	3.26	1.64	1.49
8.	42.8	9.04	47.8	64.6	91.1	9.45	3.33	1.59	1.42
9.	43.4	6.79	51.5	66.2	91.0	6.54	3.32	1.58	1.44
10.	34.4	9.16	48.9	65.5	91.3	9.82	3.34	1.26	1.15
11.	36.7	9.74	48.5	63.9	91.4	11.07	3.40	1.38	1.22
12.	41.6	8.72	37.6	68.0	92.1	8.65	3.30	1.46	1.37
13.	45.2	7.76	38.8	65.1	90.6	9.58	3.33	1.67	1.50
14.	32.8	8.64	46.4	65.7	91.3	10.66	3.45	1.20	1.09
15.	36.8	9.26	48.7	66.4	91.4	10.11	3.41	1.33	1.21
16.	38.0	9.76	51.5	65.0	90.6	10.13	3.42	1.41	1.26
17.	39.7	8.57	47.0	65.6	91.8	9.54	3.38	1.46	1.30
					100 mm. CO_2				
18.	99.8	8.46	46.2	64.8	91.6	7.36	3.06	3.71	3.30
19.	96.0	8.34	45.9	65.1	91.0	7.00	3.01	3.55	3.19
20.	99.1	8.82	46.9	65.7	90.6	7.48	3.13	3.63	3.31
21.	100.2	8.91	51.0	66.7	90.4	4.87	2.91	3.62	3.35
22.	95.4	8.05	42.4	66.5	91.2	7.02	3.07	3.45	3.16

TABLE III (continued)

EXP. NO.	pH_s	pH_c	$r_{\alpha H^+}$	$[Cl]_c$ mM./kilo H_2O	$[Cl]_s$ mM./kilo H_2O	r_{Cl}	$[HCO_3]_c$ mM./kilo H_2O	$[HCO_3]_s$ mM./kilo H_2O	r_{HCO_3}	$[\overline{CO_2}]_c$ mM./kilo H_2O	$[\overline{CO_2}]_s$ mM./kilo H_2O	$r_{\overline{CO_2}}$
					15 mm. CO_2							
1.	7.423	7.208	0.610	74.4	116.4	0.639	10.68	16.62	0.642	12.16	17.25	0.705
2.	7.423	7.198	0.596	72.7	114.8	0.633	9.26	13.79	0.671	10.45	14.31	0.730
3.	7.483	7.268	0.609	70.0	112.1	0.624	9.80	15.81	0.620	11.26	16.42	0.686
4.	7.453	7.223	0.588	70.3	116.3	0.608	9.08	13.07	0.694	10.29	13.57	0.759
					40 mm. CO_2							
5.	7.236	7.108	0.745	82.6	111.7	0.739	16.13	20.94	0.771	18.00	21.67	0.831
6.	7.223	7.078	0.716	77.6	106.2	0.731	15.59	20.04	0.777	17.12	20.73	0.826
7.	7.188	7.088	0.793	80.8	109.0	0.741	17.63	22.97	0.768	19.55	23.73	0.824
8.	7.236	7.083	0.703	80.6	110.7	0.729	17.13	22.25	0.770	18.92	23.03	0.822
9.	7.226	7.068	0.695	75.1	108.1	0.695	16.29	21.12	0.771	17.44	21.86	0.798
10.	7.242	7.090	0.704	76.4	112.8	0.677	14.39	19.56	0.736	15.96	20.83	0.789
11.	7.278	7.138	0.724	72.9	110.1	0.663	14.79	20.13	0.735	16.64	20.83	0.799
12.	7.211	7.061	-----	77.8	110.2	0.706	14.61	18.57	0.787	16.00	19.20	0.834
13.	7.231	7.061	0.678	69.3	103.9	0.667	16.41	21.90	0.749	18.15	22.67	0.801
14.	7.318	7.146	0.674	76.5	112.4	0.681	13.58	18.93	0.717	15.21	19.61	0.776
15.	7.286	7.116	0.676	78.3	110.7	0.706	13.85	18.82	0.736	15.41	19.48	0.791
16.	7.294	7.121	0.671	73.7	105.8	0.696	15.33	20.89	0.734	17.07	21.62	0.789
17.	7.266	7.102	0.685	75.5	113.0	0.669	15.51	20.34	0.769	17.15	21.06	0.814
					100 mm. CO_2							
18.	7.063	6.961	0.791	82.4	108.0	0.763	25.11	30.68	0.819	27.11	31.63	0.857
19.	7.063	6.951	0.774	82.9	109.1	0.760	24.31	29.57	0.821	26.13	30.49	0.857
20.	7.078	6.977	0.792	85.7	110.4	0.776	25.57	31.27	0.818	27.65	32.28	0.857
21.	6.953	6.858	0.803	85.5	108.0	0.791	24.27	26.55	0.914	25.51	27.36	0.933
22.	7.058	6.938	0.759	80.9	109.3	0.740	23.75	28.05	0.847	25.54	28.94	0.883

TABLE IV

RESULTS OF ANALYSES OF 1 DAY OLD BLOOD EQUILIBRATED WITH CO_2 AND H_2 AT 37° C.

EXP. NO.	p_{CO_2}	Hemoglobin	Cell Volume	Cell H_2O	Serum H_2O	Cell Carbamate	Serum Carbamate	$[H_2CO_3]_c$	$[H_2CO_3]_s$
	mm.Hg	mM. per l. blood	per cent	per cent	per cent	per cent	per cent	mM./kilo H_2O	mM./kilo H_2O
					15 mm. CO_2				
23.	20.7	9.65	48.7	64.8	90.9	22.33	3.70	0.77	0.69
24.	17.0	8.61	45.2	65.3	91.4	21.11	3.69	0.63	0.56
25.	14.6	8.93	45.1	64.4	91.9	24.78	4.01	0.55	0.48
26.	15.7	8.72	45.8	64.1	91.3	21.52	3.73	0.59	0.52
					40 mm. CO_2				
27.	36.7	8.56	46.6	65.3	91.1	17.85	3.50	1.35	1.22
28.	38.6	8.67	47.6	65.8	90.5	17.53	3.45	1.41	1.29
29.	33.4	9.16	48.3	65.4	91.5	18.62	3.45	1.22	1.11
30.	42.9	9.60	50.8	66.8	91.7	16.93	3.42	1.55	1.42
31.	38.7	9.62	48.0	65.9	91.6	18.44	3.47	1.41	1.28
32.	37.4	9.74	47.9	64.4	91.3	18.87	3.46	1.39	1.24
					100 mm. CO_2				
33.	95.3	9.60	51.4	67.0	91.8	9.56	3.08	3.42	3.14
34.	95.8	9.62	48.6	66.7	91.5	10.55	3.11	3.45	3.17
35.	102.1	9.64	50.5	66.6	90.8	11.00	3.09	3.69	3.40
36.	104.8	9.64	50.6	66.3	90.9	10.86	3.11	3.80	3.49

TABLE IV (continued)

EXP. NO.	pH_s	pH_c	$\frac{r}{\alpha_{H^+}}$	$[Cl]_c$ mM./kilo H_2O	$[Cl]_s$ mM./kilo H_2O	$\frac{r}{Cl}$	$[HCO_3]_c$ mM./kilo H_2O	$[HCO_3]_s$ mM./kilo H_2O	$\frac{r}{HCO_3}$	$[\overline{CO_2}]_c$ mM./kilo H_2O	$[\overline{CO_2}]_s$ mM./kilo H_2O	$\frac{r}{\overline{CO_2}}$
					15 mm. CO_2							
23.	7.478	7.283	0.641	78.4	113.2	0.692	11.72	18.52	0.633	15.10	19.24	0.785
24.	7.473	7.263	0.617	76.3	112.5	0.678	10.02	15.67	0.640	12.71	16.27	0.781
25.	7.580	7.373	0.621	71.2	111.0	0.642	9.44	16.42	0.575	12.56	17.11	0.734
26.	7.503	7.278	0.596	71.9	113.6	0.633	9.74	14.59	0.666	12.38	15.17	0.816
					40 mm. CO_2							
27.	7.343	7.155	0.647	79.2	111.9	0.707	16.65	23.25	0.716	20.27	24.10	0.841
28.	7.308	7.148	0.692	79.7	106.1	0.751	15.90	21.45	0.741	19.28	22.22	0.867
29.	7.315	7.168	0.713	76.9	108.6	0.717	15.43	21.23	0.727	18.97	22.00	0.862
30.	7.293	7.118	0.669	79.8	109.8	0.726	17.26	23.87	0.723	20.78	24.72	0.841
31.	7.323	7.138	0.653	81.1	110.4	0.734	16.64	22.98	0.724	20.41	23.81	0.857
32.	7.326	7.142	0.654	75.9	108.3	0.701	16.08	22.46	0.716	19.83	23.27	0.853
					100 mm. CO_2							
33.	7.068	6.928	0.725	85.5	106.1	0.806	26.91	31.14	0.864	29.77	32.13	0.927
34.	7.088	6.938	0.708	85.6	106.2	0.815	27.19	31.92	0.852	30.40	32.95	0.922
35.	7.073	6.958	0.767	85.9	105.0	0.818	28.17	33.12	0.850	31.66	34.20	0.925
36.	7.068	6.953	0.767	85.8	104.9	0.818	28.66	33.18	0.864	32.15	34.25	0.939

results of which are recorded in Table VII, only oxygenated blood, one day old, at a CO_2 tension of approximately 40 mm., was used. These experiments involved a study of the effects upon the electrolyte distribution in blood of alteration of the chloride or bicarbonate concentration, and of the addition of isotonic sucrose solution.

Alteration of chloride and bicarbonate concentration - For experiments in which chloride was to be increased, the fresh blood was centrifuged, a portion of the serum was removed, and approximately 100 milliequivalents per liter of NaCl were added. For the experiments in which bicarbonate was to be increased, approximately 30 milliequivalents per liter of Na_2CO_3 were added to a portion of serum. For experiments in which bicarbonate or chloride was to be removed, 12 ml. of serum, approximately half of the serum in a 45 ml. sample of blood, was dialyzed in a cellophane bag against two 12-liter portions of distilled water for one hour each. When low bicarbonate was desired, Ca^{++}, Mg^{++}, and $H_2PO_4^-$ were added to the dialyzed serum in amount sufficient to give normal concentrations, and enough NaCl was added so that the total salt concentration was again approximately 160 milliequivalents per liter. When low chloride was desired, the Ca^{++}, Mg^{++}, and $H_2PO_4^-$ were added, and enough Na_2CO_3 was added so that a total salt concentration of 160 milliequivalents per liter was present. The globulin which had come out of solution, due to the removal of salts by dialysis, was redissolved. The "artificial serum" was then returned to the remainder of the blood sample.

Dilution of serum with isotonic sucrose solution - For the sucrose experiments, the serum was diluted with approximately an equal volume of ten per cent sucrose solution. In all cases, the treated blood was well mixed and allowed to stand in the refrigerator overnight. Control experiments were run with untreated blood.

TABLE V

RESULTS OF ANALYSES OF FRESH BLOOD EQUILIBRATED WITH CO_2 AND AIR AT 37° C.

EXP. NO.	p_{CO_2}	Hemoglobin	Cell Volume	Cell H_2O	Serum H_2O	Cell Carbamate	Serum Carbamate	$[H_2CO_3]_c$	$[H_2CO_3]_s$
	mm. Hg	mM. per l. blood	per cent	per cent	per cent	per cent	per cent	mM./kilo H_2O	mM./kilo H_2O
				15 mm. CO_2					
37.	17.9	8.85	47.2	67.1	91.5	14.05	3.89	0.48	0.45
38.	13.6	8.50	44.6	65.6	91.6	12.09	3.71	0.66	0.59
				40 mm. CO_2					
39.	41.0	9.65	49.7	65.0	91.2	11.11	3.42	1.52	1.36
40.	35.4	7.76	41.1	67.2	91.4	11.04	3.44	1.27	1.18
				100 mm. CO_2					
41.	97.7	9.51	49.2	67.6	90.1	7.04	3.10	3.48	3.28
42.	88.7	10.05	55.4	68.1	91.1	7.09	3.16	3.13	2.94

TABLE V (continued)

EXP. NO.	pH_s	pH_c	$\frac{r}{\alpha_{H^+}}$	$[Cl]_c$	$[Cl]_s$	$\frac{r}{Cl}$	$[HCO_3]_c$	$[HCO_3]_s$	$\frac{r}{HCO_3}$	$[\overline{CO_2}]_c$	$[\overline{CO_2}]_s$	$\frac{r}{\overline{CO_2}}$
				mM./kilo H_2O	mM./kilo H_2O		mM./kilo H_2O	mM./kilo H_2O		mM./kilo H_2O	mM./kilo H_2O	
15 mm. CO_2												
37.	7.616	7.355	0.547	63.3	108.4	0.584	10.51	16.80	0.625	12.23	17.48	0.700
38.	7.489	7.224	0.543	68.0	113.8	0.597	10.84	16.20	0.669	12.34	16.82	0.733
40 mm. CO_2												
39.	7.295	7.148	0.716	72.2	109.6	0.659	17.86	24.90	0.717	20.10	25.78	0.780
40.	7.308	7.174	0.734	72.6	110.5	0.657	14.42	19.94	0.723	16.23	20.65	0.786
100 mm. CO_2												
41.	7.083	6.936	0.713	83.3	105.3	0.791	26.62	31.53	0.848	28.79	32.55	0.884
42.	7.118	6.958	0.692	74.4	101.6	0.732	25.97	31.70	0.819	27.97	32.73	0.855

TABLE VI

RESULTS OF ANALYSES OF FRESH BLOOD EQUILIBRATED WITH CO_2 AND H_2 AT 37° C.

EXP. NO.	P_{CO_2}	Hemoglobin	Cell Volume	Cell H_2O	Serum H_2O	Cell Carbamate	Serum Carbamate	$[H_2CO_3]_c$	$[H_2CO_3]_s$
	mm.Hg	mM.per l. blood	per cent	per cent	per cent	per cent	per cent	mM./kilo H_2O	mM./kilo H_2O
				15 mm. CO_2					
43.	13.4	8.85	46.5	67.1	91.4	24.23	4.02	0.48	0.44
44.	16.1	8.50	43.9	65.6	91.4	22.20	4.86	0.59	0.53
				40 mm. CO_2					
45.	40.1	9.65	49.2	65.1	91.1	18.93	3.47	1.48	1.33
46.	36.5	7.76	40.6	66.9	91.4	18.02	3.48	1.31	1.21
				100 mm. CO_2					
47.	92.4	9.51	48.7	67.6	89.9	11.80	3.18	3.29	3.09
48.	87.9	10.05	55.0	68.3	91.0	10.59	3.21	3.10	2.92

TABLE VI (continued)

EXP. NO.	pH_s	pH_c	$\frac{r}{-\alpha H^+}$	$[Cl]_c$	$[Cl]_s$	$\frac{r}{-Cl}$	$[HCO_3]_c$	$HCO_3{}_s$	$\frac{r}{-HCO_3}$	$[\overline{CO_2}]_c$	$[\overline{CO_2}]_s$	$\frac{r}{-\overline{CO_2}}$
				mM./kilo H_2O	mM./kilo H_2O		mM./kilo H_2O	mM./kilo H_2O		mM./kilo H_2O	mM./kilo H_2O	
15 mm. CO_2												
37.	7.698	7.383	0.484	62.9	107.2	0.587	10.92	18.77	0.582	14.42	19.56	0.737
38.	7.583	7.295	0.516	70.4	112.7	0.627	10.91	17.25	0.632	14.02	17.94	0.782
40 mm. CO_2												
39.	7.328	7.195	0.736	76.0	107.4	0.708	19.47	27.11	0.718	24.02	28.09	0.855
40.	7.336	7.156	0.662	78.6	109.4	0.719	15.92	22.18	0.717	19.41	20.98	0.845
100 mm. CO_2												
41.	7.131	6.970	0.691	83.4	102.9	0.811	28.63	33.80	0.848	32.48	34.91	0.930
42.	7.158	6.961	0.648	75.6	100.2	0.754	27.88	34.02	0.820	31.19	35.17	0.887

TABLE VII

RESULTS OF ANALYSES OF 1 DAY OLD BLOOD IN WHICH CHLORIDE OR BICARBONATE CONCENTRATION HAS BEEN ALTERED. (BLOOD EQUILIBRATED WITH 40 mm. CO_2 AND AIR AT 37° C.)

EXP. NO.	p_{CO_2}	Hemoglobin	Cell Volume	Cell H_2O	Serum H_2O	Cell Carbamate	Serum Carbamate	$[H_2CO_3]_c$	$[H_2CO_3]_s$
	mm.Hg	mM.per l. blood	per cent	per cent	per cent	per cent	per cent	mM./kilo H_2O	mM./kilo H_2O
					Low Bicarbonate				
49.	32.8	8.64	46.4	65.7	91.3	10.66	3.45	1.20	1.09
50.	36.8	9.26	48.7	66.4	91.4	10.11	3.41	1.33	1.21
51.	27.9	8.64	47.6	66.3	91.8	8.58	3.26	1.01	0.92
52.	33.4	9.26	48.8	65.4	91.9	8.67	3.21	1.23	1.10
53.	34.7	8.68	48.3	64.9	91.8	8.45	3.24	1.29	1.14
					Low Chloride				
54.	38.0	9.76	51.5	65.0	90.6	10.13	3.42	1.41	1.26
55.	39.7	8.57	47.0	65.6	91.8	9.54	3.38	1.46	1.30
56.	38.5	9.76	49.3	64.5	91.4	15.90	3.99	1.44	1.24
57.	33.0	8.57	45.2	66.0	92.5	15.47	4.04	1.20	1.08
58.	32.4	8.68	45.8	66.1	91.8	15.46	4.01	1.18	1.07
					High Bicarbonate				
59.	45.2	7.76	38.8	65.1	90.6	9.38	3.33	1.67	1.50
60.	48.1	7.76	35.9	63.3	90.9	12.95	3.62	1.83	1.59
61.	31.0	7.20	37.5	64.8	91.8	13.35	3.82	1.15	1.02
62.	33.5	7.20	37.9	64.8	91.8	12.96	3.78	1.24	1.10
					High Chloride				
63.	41.6	6.72	37.6	68.0	92.1	8.65	3.30	1.46	1.37
64.	40.7	6.72	31.4	62.4	92.4	10.10	3.28	1.55	1.33
65.	38.3	8.80	42.2	59.9	92.0	11.46	3.39	1.51	1.25
66.	38.2	8.80	42.1	60.2	91.7	11.32	3.39	1.50	1.25
					Isotonic Sucrose Solution Added				
67.	35.9	5.58	27.5	62.0	91.5	10.88	1.57	1.39	1.18
68.	36.3	5.58	27.5	61.9	91.5	11.43	1.59	1.41	1.20

TABLE VII (continued)

EXP. NO.	pH_s	pH_c	$r_{\alpha H^+}$	$[Cl]_c$ mM./kilo H_2O	$[Cl]_s$ mM./kilo H_2O	r_{Cl}	$[HCO_3]_c$ mM./kilo H_2O	$[HCO_3]_s$ mM./kilo H_2O	r_{HCO_3}	$[\overline{CO_2}]_c$ mM./kilo H_2O	$[\overline{CO_2}]_s$ mM./kilo H_2O	$r_{\overline{CO_2}}$
				Low Bicarbonate								
49.	7.318	7.146	0.674	76.5	112.4	0.681	13.58	18.93	0.715	15.21	19.61	0.776
50.	7.286	7.116	0.676	78.3	110.7	0.706	13.85	18.82	0.736	15.41	19.48	0.791
51.	7.188	7.048	0.723	88.5	124.8	0.709	9.84	12.90	0.763	10.76	13.34	0.807
52.	7.160	7.032	0.745	91.9	126.8	0.724	11.53	14.51	0.795	12.63	14.98	0.843
53.	7.178	7.045	-----	92.3	129.9	0.716	12.52	15.68	0.798	13.67	16.20	0.843
				Low Chloride								
54.	7.294	7.121	0.671	73.7	105.8	0.696	15.33	20.89	0.734	17.07	21.62	0.789
55.	7.266	7.102	0.685	75.5	113.0	0.669	15.51	20.34	0.762	17.15	21.06	0.814
56.	7.678	7.432	0.567	42.3	74.7	0.566	26.03	50.70	0.513	30.97	52.83	0.586
57.	7.718	7.455	0.545	42.6	75.0	0.568	26.12	48.06	0.543	30.90	50.06	0.617
58.	7.691	7.445	-----	46.0	78.6	0.585	23.47	43.98	0.533	27.76	45.82	0.606
				High Bicarbonate								
59.	7.231	7.061	0.678	69.3	103.9	0.667	16.41	21.90	0.749	18.15	22.67	0.801
60.	7.432	7.203	0.590	64.7	105.5	0.613	21.44	34.78	0.617	24.64	36.09	0.683
61.	7.569	7.306	0.547	65.8	109.8	0.598	19.28	32.86	0.587	22.27	34.17	0.651
62.	7.538	7.287	0.561	64.4	109.4	0.588	19.55	32.53	0.601	22.47	33.82	0.664
				High Chloride								
63.	7.211	7.061	-----	77.8	110.2	0.706	14.61	18.57	0.787	16.00	19.20	0.834
64.	7.198	7.058	-----	126.4	181.7	0.696	14.24	18.48	0.770	15.84	19.11	0.829
65.	7.270	7.132	0.727	118.2	175.2	0.675	15.05	20.36	0.740	17.01	21.07	0.808
66.	7.272	7.126	0.716	119.7	177.2	0.675	14.81	20.25	0.731	16.71	20.97	0.797
				Isotonic Sucrose Solution Added								
67.	7.069	7.114	1.109	68.5	59.5	1.151	15.13	12.36	1.226	17.03	12.55	1.356
68.	7.098	7.119	1.050	68.0	59.3	1.147	15.28	12.47	1.225	17.14	12.67	1.352

Results and Discussion

The results of the experimental work are best described graphically as shown in Figs. 3 to 11. The values in Tables III to VII of the distribution ratios for $[\alpha_{H^+}]$, $[Cl]$, $[HCO_3]$, and $[\overline{CO}_2]$ are plotted against pH of the serum. The straight lines represent the best lines which can be fitted to the experimental points. Figs. 3, 4, 5, and 6 illustrate the results with normal blood, i.e., without the addition or removal of chloride, bicarbonate, or sucrose. The results for fresh blood, in which glycolysis was active, are plotted with the results for blood one day old, in which glycolysis was at a minimum. Because a closer approximation of true values for the pH of the cells from fresh blood is desirable, the values for $\underline{r}_{\alpha_{H^+}}$ are not included. Although agreement is only fair in the alkaline pH range, almost all of the points fall within an experimental error range. Therefore, it seems evident that glycolysis has no effect upon the distribution of diffusible ions across the red blood cell membrane, at least insofar as for those ions tested.

Fig. 7 represents a composite of Figs. 3, 4, 5, and 6, together with the calculated curves of Van Slyke, Wu, and McLean (55). The following facts may be observed from this graph:

1. There is fair agreement for the slopes of the curves for $[\alpha_{H^+}]$, $[Cl]$, and $[\overline{CO}_2]$ in reduced blood (broken lines).

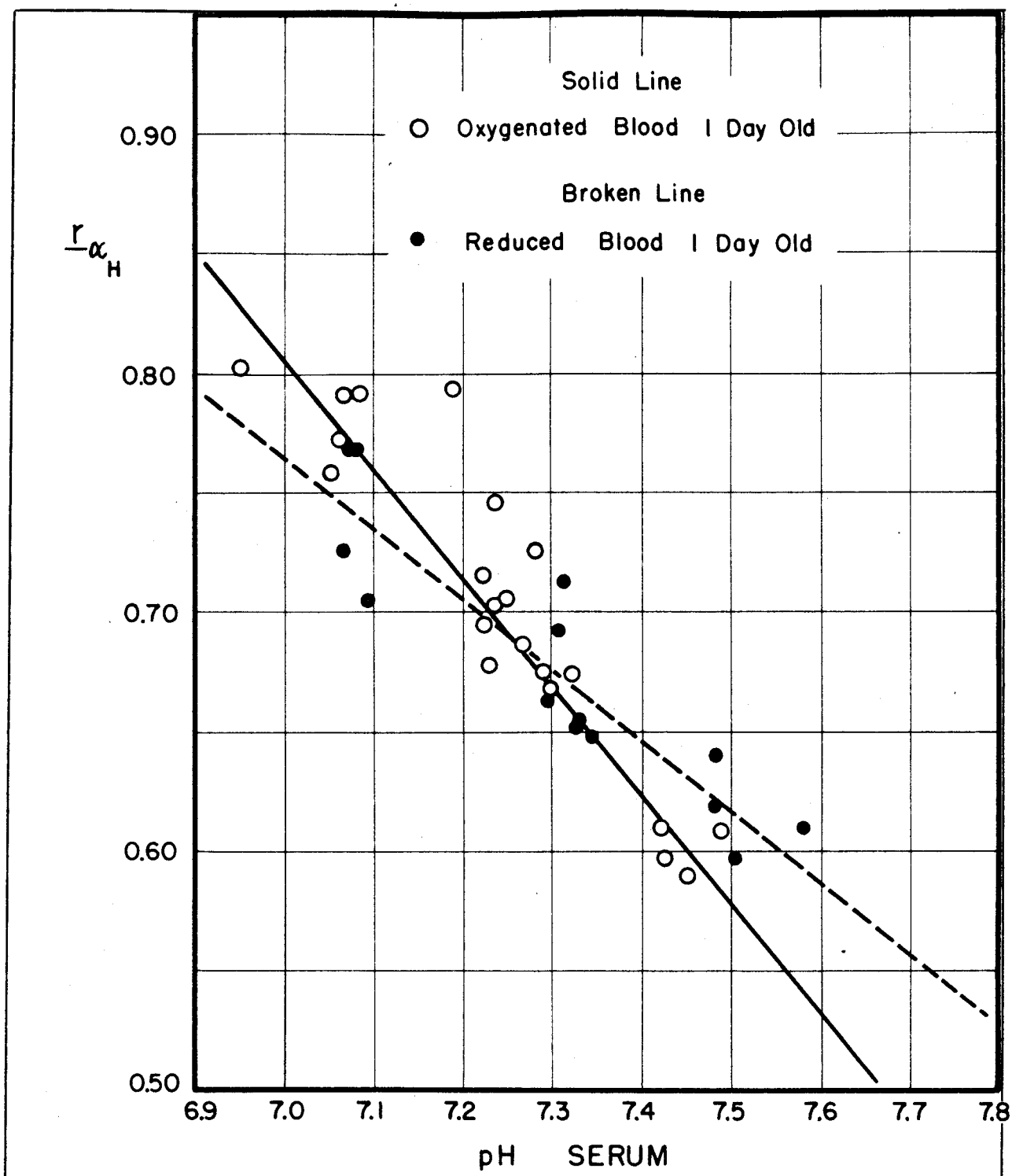

Fig. 3. The change in the distribution ratio for $[\alpha_{H^+}]$ with change in serum pH

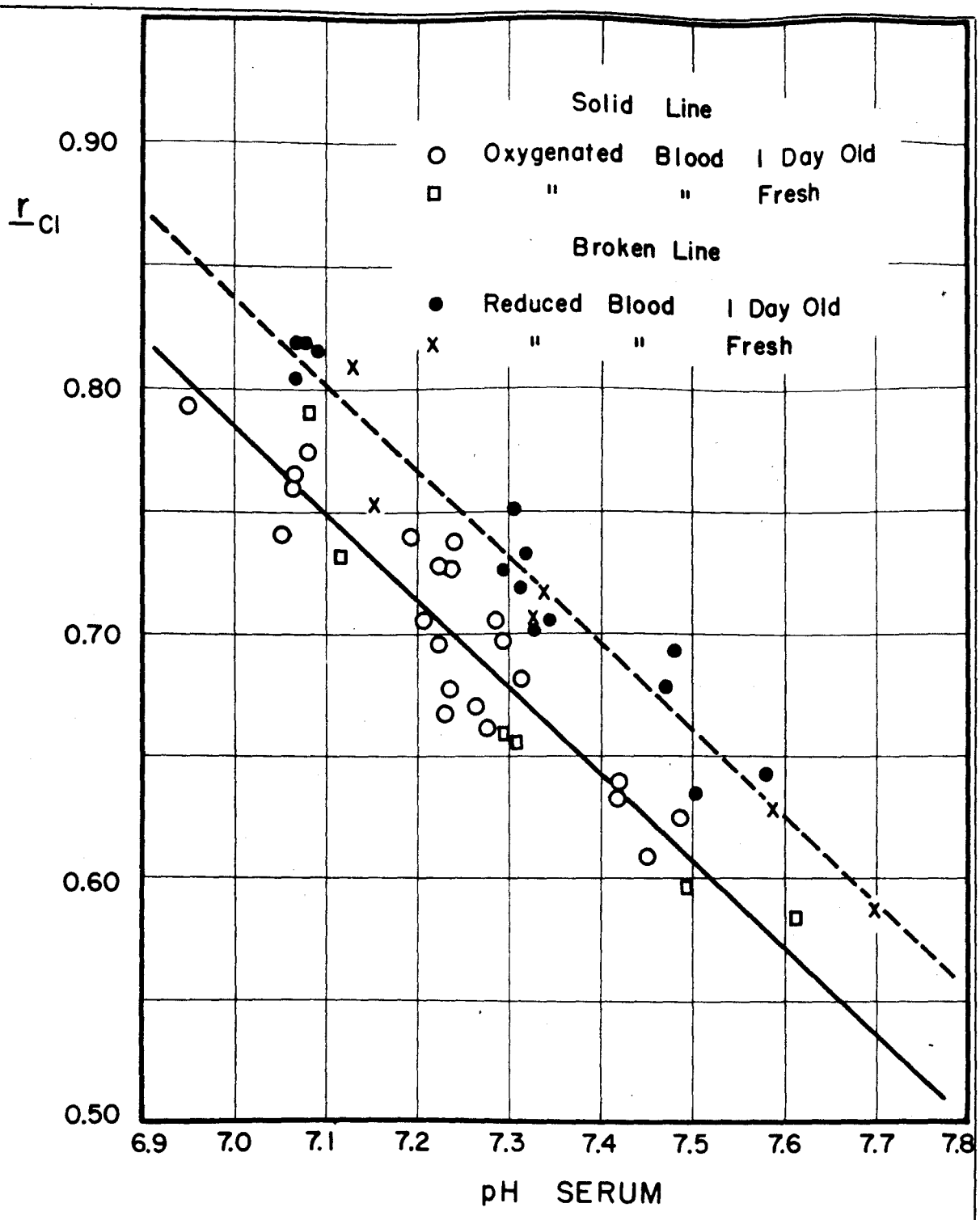

Fig. 4. The change in the distribution ratio for [Cl] with change in serum pH.

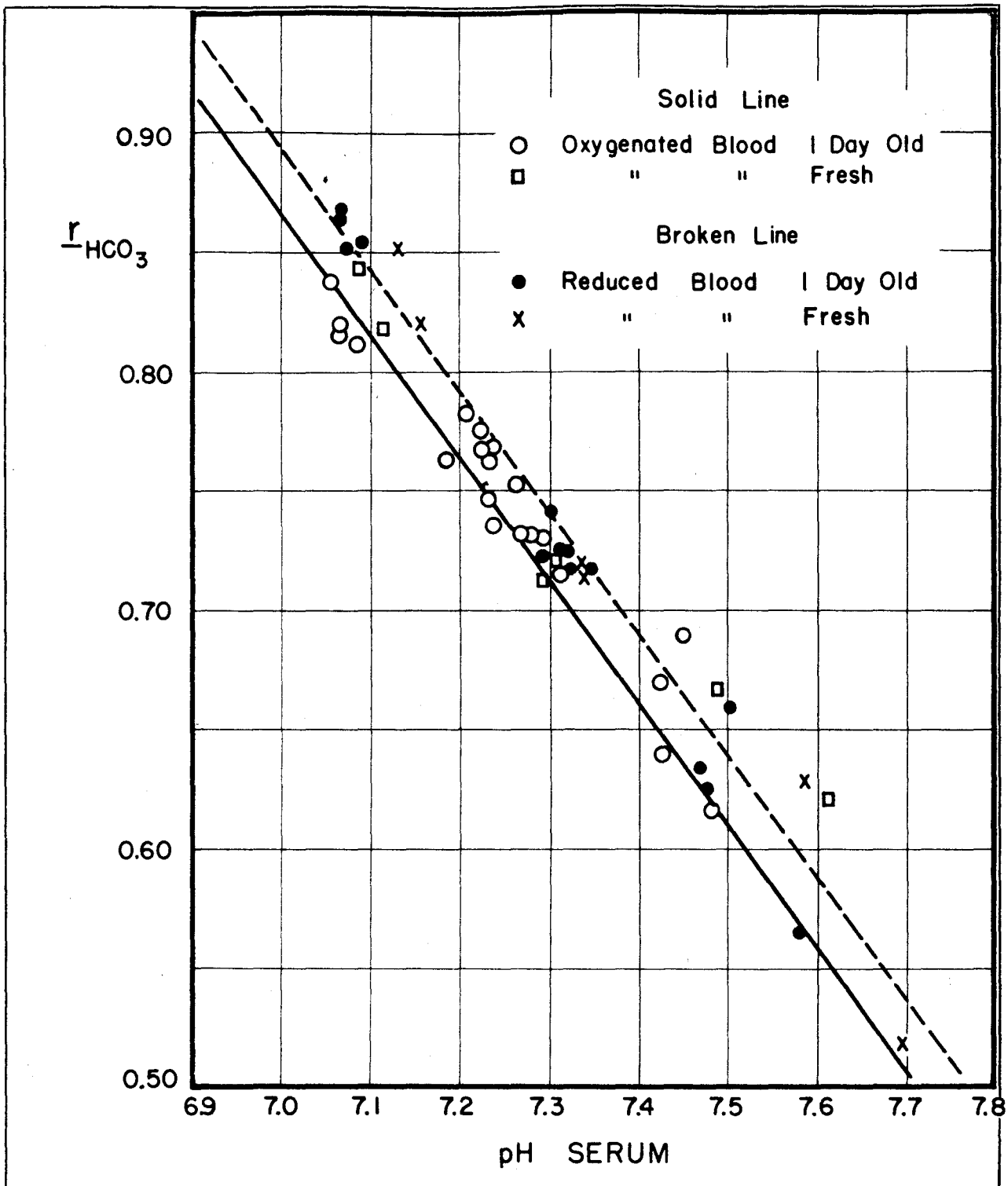

Fig. 5. The change in the distribution ratio for $[HCO_3]$ with change in serum pH.

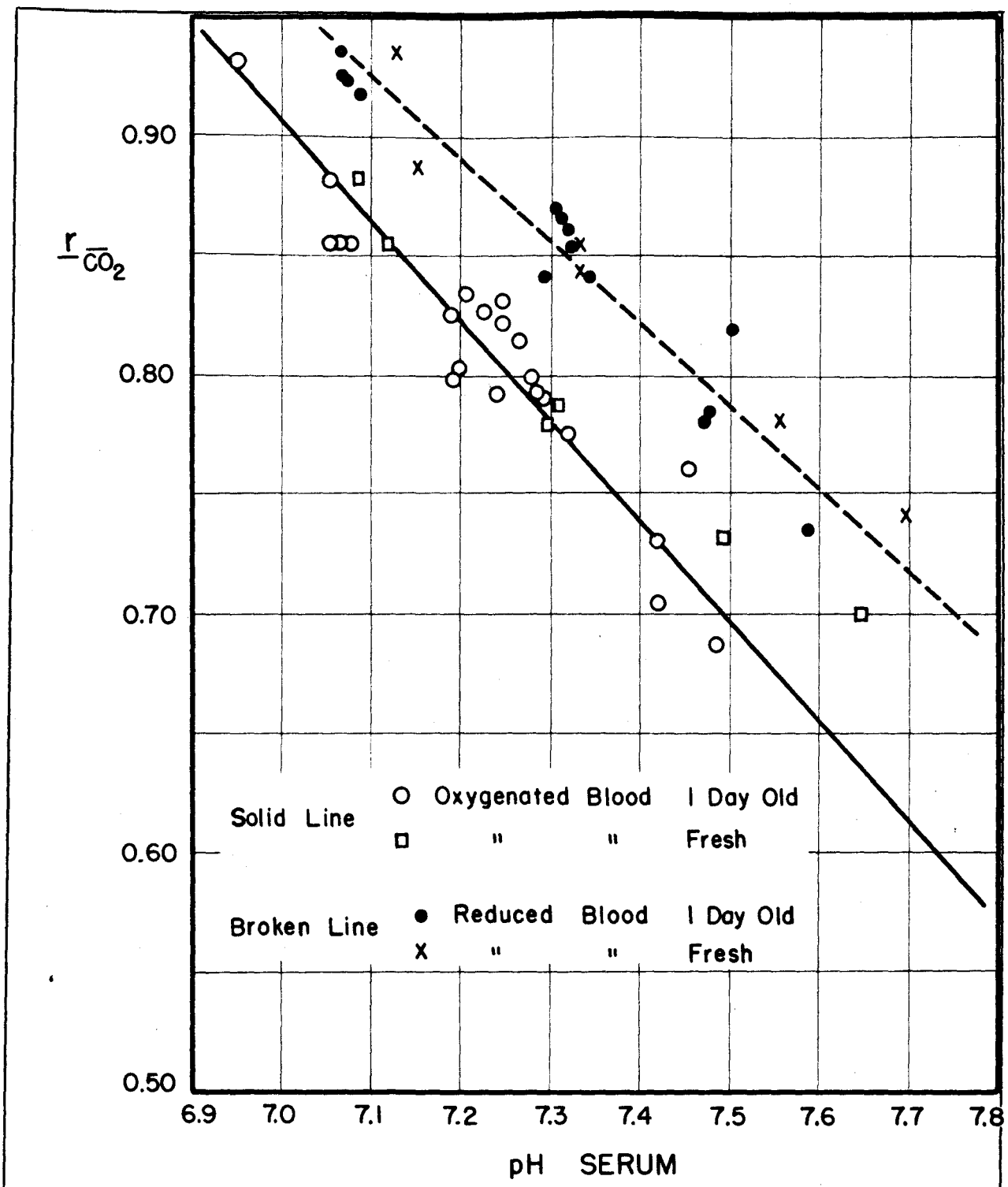

Fig. 6. The change in the distribution ration for $[\overline{CO}_2]$ with change in serum pH.

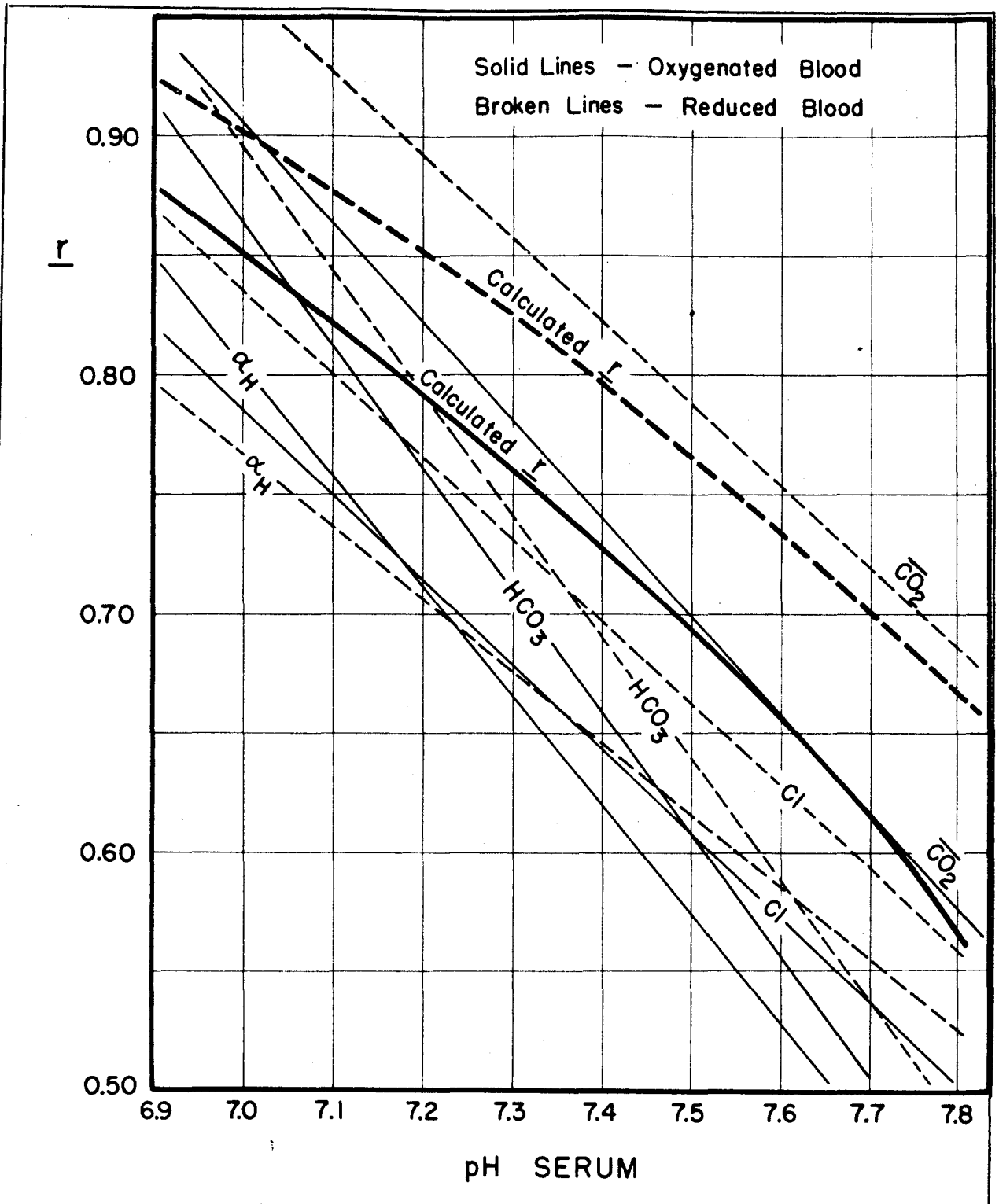

Fig. 7. Comparison of observed curves for $[\alpha_{H^+}]$, [Cl], $[HCO_3]$, and $[\overline{CO_2}]$, with curves of the Van Slyke group from calculated values of r (1923) (1925)

2. The curve for $[Cl]$ in oxygenated blood (solid lines) is approximately parallel to the curve for reduced blood.

3. The curves for $[\alpha H^+]$ and $[\overline{CO_2}]$ in oxygenated blood (solid lines) are approximately parallel but are not parallel to the curves for reduced blood.

4. The curves found for $[\alpha H^+]$, $[Cl]$, and $[\overline{CO_2}]$ in reduced blood are in good agreement with the calculated curves of Van Slyke's group (55). The distance between the curves for $[Cl]$ in oxygenated and reduced blood, representing the effect upon the distribution ratios of the greater base-binding power of oxygenated hemoglobin, approximates that predicted by Van Slyke, Wu, and McLean. This is also true of the curves for $[\overline{CO_2}]$. In the case of $[\alpha H^+]$, the curves for reduced and oxygenated blood intersect. This is in agreement with the results of Dill, Edwards, and Consolazio, who calculated pH from their values for combined-CO_2 and H_2CO_3.

5. The curves for $[HCO_3]$ in oxygenated and reduced blood are approximately parallel, but are not parallel to the other curves.

6. The curves for $[HCO_3]$ are not in agreement with the prediction of Van Slyke, Wu, and McLean for the effect of oxygenation of the hemoglobin. The distance between the two curves is too small.

In order to have conformation with Donnan's law, the thermodynamic activity ratios of the diffusible ions must be equal, so that

(41) $$\frac{[\alpha H^+]_s}{[\alpha H^+]_c} = \frac{[\alpha Cl^-]_c}{[\alpha Cl^-]_s} = \frac{[\alpha HCO_3^-]_c}{[\alpha HCO_3^-]_s} = \frac{[\alpha \overline{CO_2}]_c}{[\alpha \overline{CO_2}]_s}$$

Since the concentration of Cl, HCO_3, and $\overline{CO}_2$ has been found in terms of molality, activity coefficients, , must be introduced for these ions:

(42) $$\frac{[\alpha H^+]_s}{[\alpha H^+]_c} = \frac{\gamma Cl_c\,[Cl]_c}{\gamma Cl_s\,[Cl]_s} = \frac{\gamma HCO_{3c}[HCO_3]_c}{\gamma HCO_{3s}[HCO_3]_s} = \frac{\gamma\,\overline{CO}_{2c}[\overline{CO}_2]_c}{\gamma\,\overline{CO}_{2s}[\overline{CO}_2]_s}$$

Differences in γ for cells and serum will thus result in deviations from equality when molal concentrations are used in calculating the ratios of the distribution of the diffusible ions. The value for $\frac{\gamma Cl_c}{\gamma Cl_s}$ may be obtained by dividing each $\frac{[\alpha H^+]_s}{[\alpha H^+]_c}$ value by the $\frac{[Cl]_c}{[Cl]_s}$ value for the same pH and degree of oxygenation. The $\frac{\gamma HCO_{3\,c}}{\gamma HCO_{3\,s}}$ and $\frac{\gamma\,\overline{CO}_{2\,c}}{\gamma\,\overline{CO}_{2\,s}}$ values may be obtained similarly. When this is done, for reduced blood at a serum pH of 7.4, the relation above becomes

(43) $$\frac{[\alpha H^+]_s}{[\alpha H^+]_c} = 0.93\,\frac{[Cl]_c}{[Cl]_s} = 0.94\,\frac{[HCO_3]_c}{[HCO_3]_s} = 0.80\,\frac{[\overline{CO}_2]_c}{[\overline{CO}_2]_s}$$

which is in agreement with the calculated values of Rapoport and Guest (35). From this it is seen that by applying the carbamate corrections, the $[HCO_3]$ results obtained are in good accord with those demanded by Donnan's law. However, the ratio of the activity coefficients for bicarbonate will not be consistent with variations in pH, due to the difference in the slopes of the curves for $[HCO_3]$ and $[\alpha H^+]$ in reduced blood. The ratio of the activity coefficients for total bound CO_2 will be consistent with variations in pH.

Its low value indicates either that there is in cells an inactive part, presumably the carbamate, or that in the cells γ for total combined-CO_2 is less than for Cl. The distribution ratios of $[Cl]$ and $[\overline{CO_2}]$ are related as follows:

(44) $$\frac{[Cl]_c}{[Cl]_s} = 0.86 \frac{[\overline{CO_2}]_c}{[\overline{CO_2}]_s}$$

This is in agreement with the findings of Hastings, Sendroy, McIntosh, and Van Slyke (21).

The fact that the curves for $[\alpha H^+]$ and $[\overline{CO_2}]$ in oxygenated blood have slopes different from those for reduced blood, but are approximately parallel to one another suggests that the activity coefficient for the total bound CO_2 is different for oxygenated and reduced blood, and that this difference is reflected in hydrogen ion activity measurements. This could account for the fact that the curves for $[\alpha H^+]$ in oxygenated and reduced blood intersect.

The possibility that the calculations for bicarbonate are, in part, incorrect, seem to be suggested by the following facts:

1. The slopes of the curves for $[HCO_3]$ in reduced and oxygenated blood are different from those for the other blood constituents studied.
2. The non-agreement of these two curves with the prediction of Van Slyke, Wu, and McLean (55) for the effect of oxygenation of the hemoglobin.
3. The apparent non-relationship of $[HCO_3]$ and $[\alpha H^+]$, as indicated by the fact that the oxygenation of the hemoglobin does not change the slope

for $[HCO_3]$, but does change the slope of the curve for $[\alpha H^+]$. Stadie and O'Brien (41) determined their values for K_{Am} by using dialyzed hemoglobin solutions to which were added known amounts of $NaHCO_3$ and NaCl. Thus, they were able to calculate Hb^- by the equation

(45) $$(Hb^-) = (B^+) - (Hb_{Am}-) - (HCO_3-)$$

(B^+), the available base, is the total base less chloride. $(Hb_{Am}-)$ was determined. (HCO_3^-) is found by subtracting $(Hb_{Am}-)$ from the total bound CO_2, which was determined. In cell hemolysates, there are present anions other than chloride and bicarbonate. The amount of these anions must be known in order to determine (B^+) and, thus, calculate (Hb^-) from equation (42). Because an accurate estimation of these anions is difficult, the equations of Dill, Edwards, and Consolazio (9) were used for calculating (Hb^-) in cell hemolysates. This method of determining (Hb^-) might be a source of error in the calculations for bicarbonate.

Figs. 8, 9, 10, and 11 illustrate the results of the experiments involving the alteration of the bicarbonate or chloride concentration. The solid line represents the best straight line found for the results with normal oxygenated blood. The experimental points approximate the path of the normal line within the limits of the range of experimental error. Thus, the experiments in which bicarbonate was increased or decreased show that the response of $\underline{r}_{\overline{CO_2}}$ to a given change of the serum pH is the same if the change is produced by altering bicarbonate as it is when CO_2 tension is varied. The change of $\underline{r}_{\alpha H^+}$, $\underline{r}_{Cl}$, or $\underline{r}_{HCO_3}$ with serum pH is also independent of the

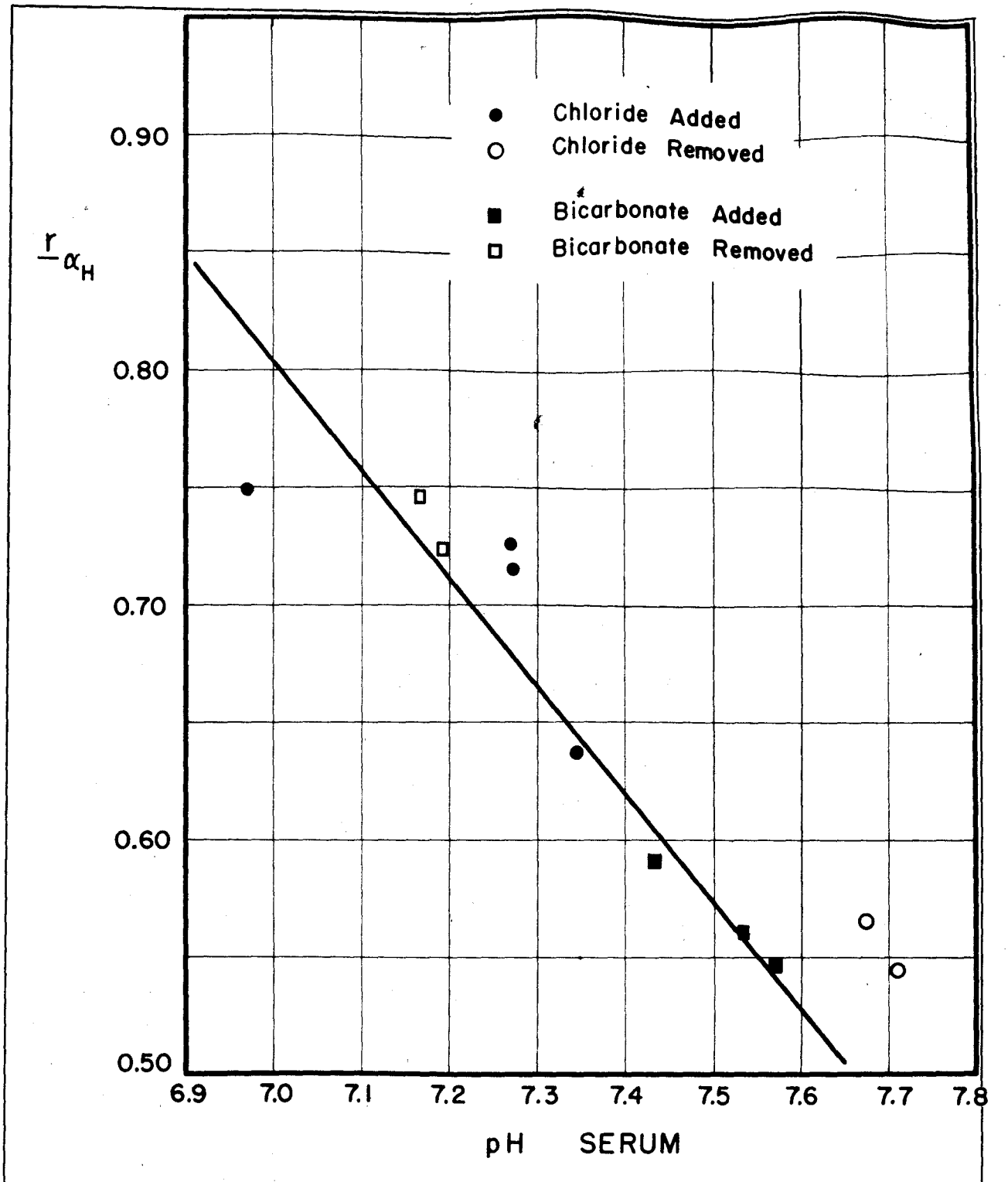

Fig. 8. The effect of alteration of the chloride or bicarbonate concentration upon the distribution ratio for $[\alpha_{H^+}]$. The solid line represents the observed values for normal oxygenated blood.

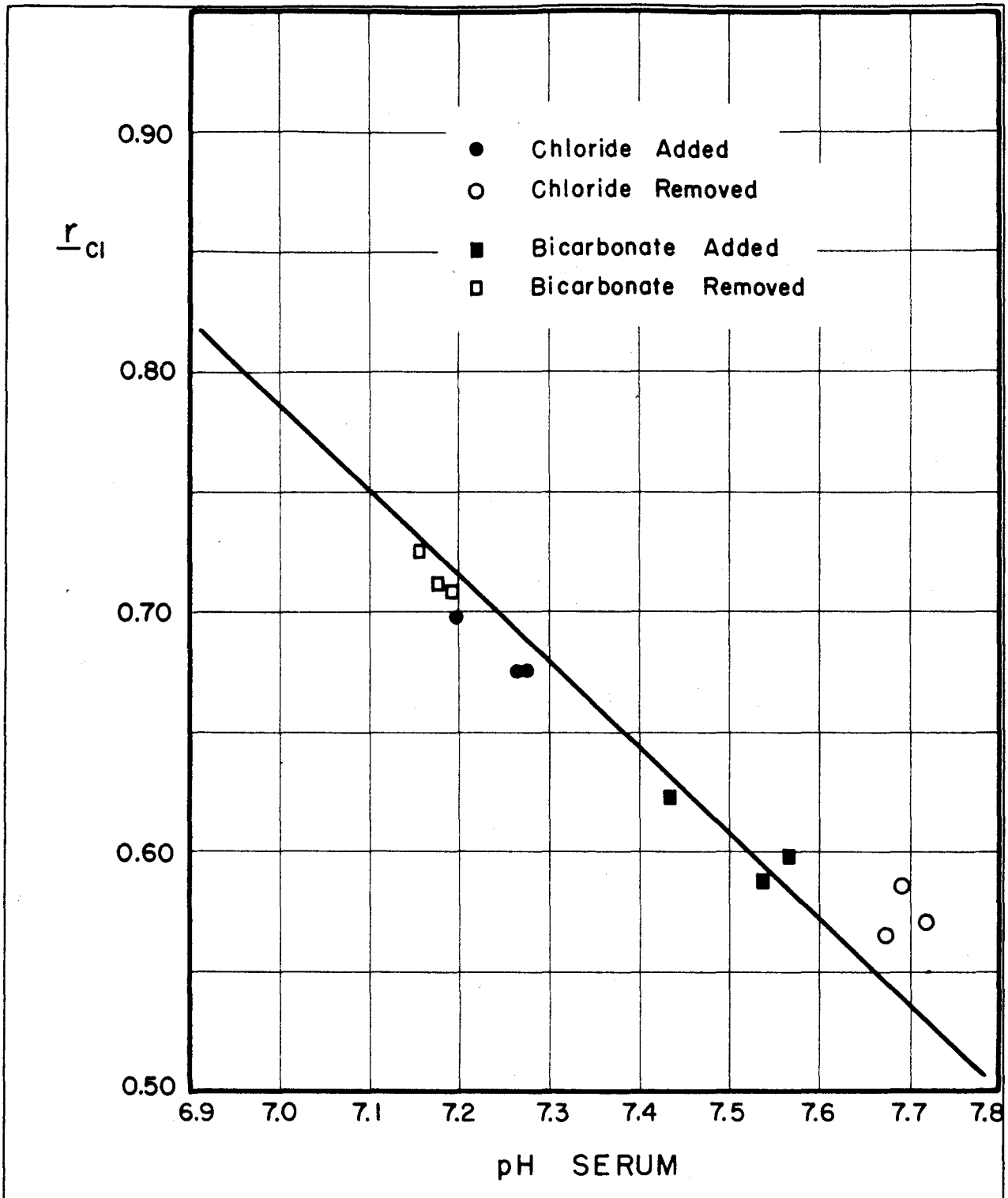

Fig. 9. The effect of alteration of the chloride or bicarbonate concentration upon the distribution ratio for [Cl]. The solid line represents the observed values for normal oxygenated blood.

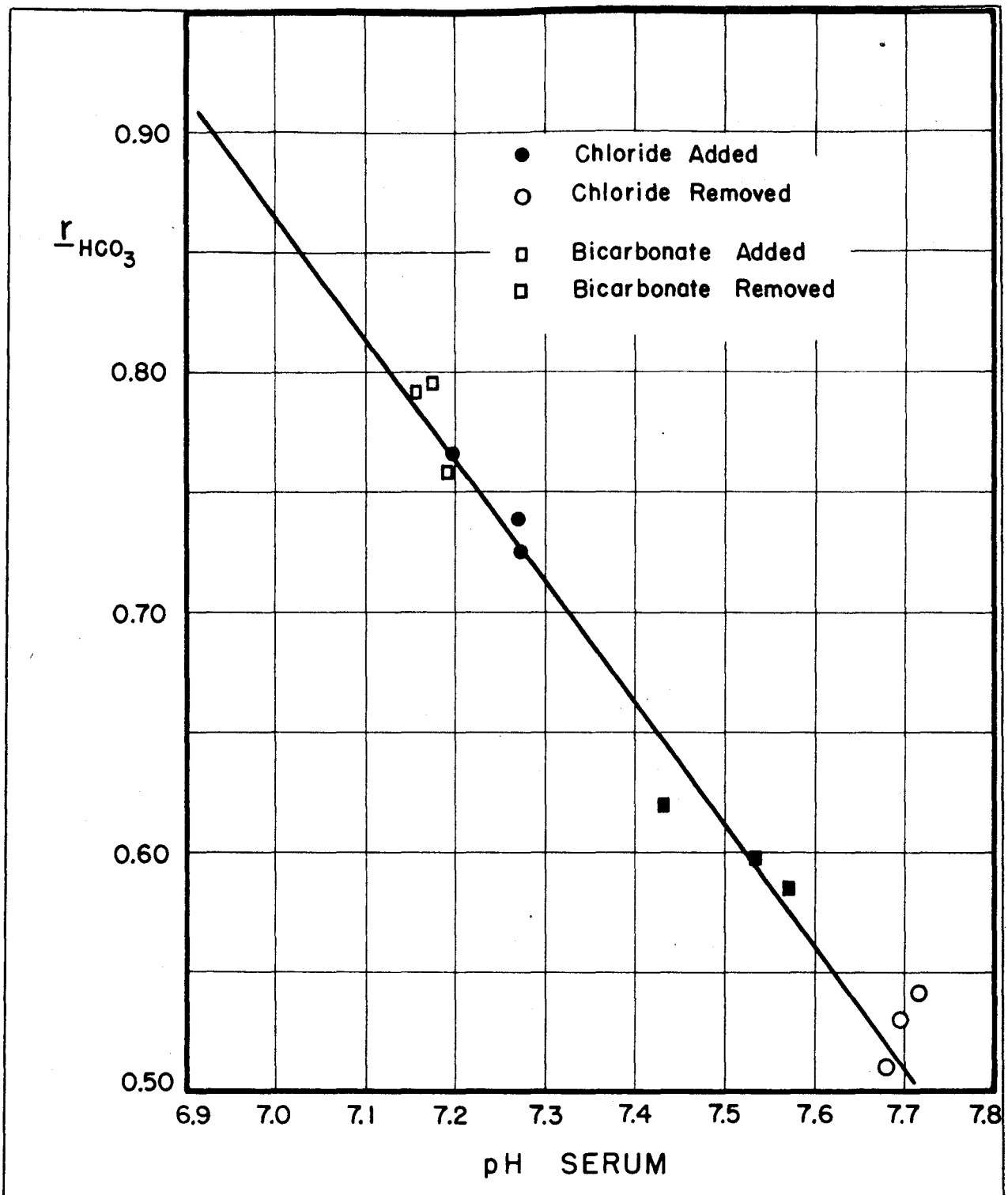

Fig. 10. The effect of alteration of the chloride or bicarbonate concentration upon the distribution ratio for $[HCO_3]$. The solid line represents the observed values for normal oxygenated blood.

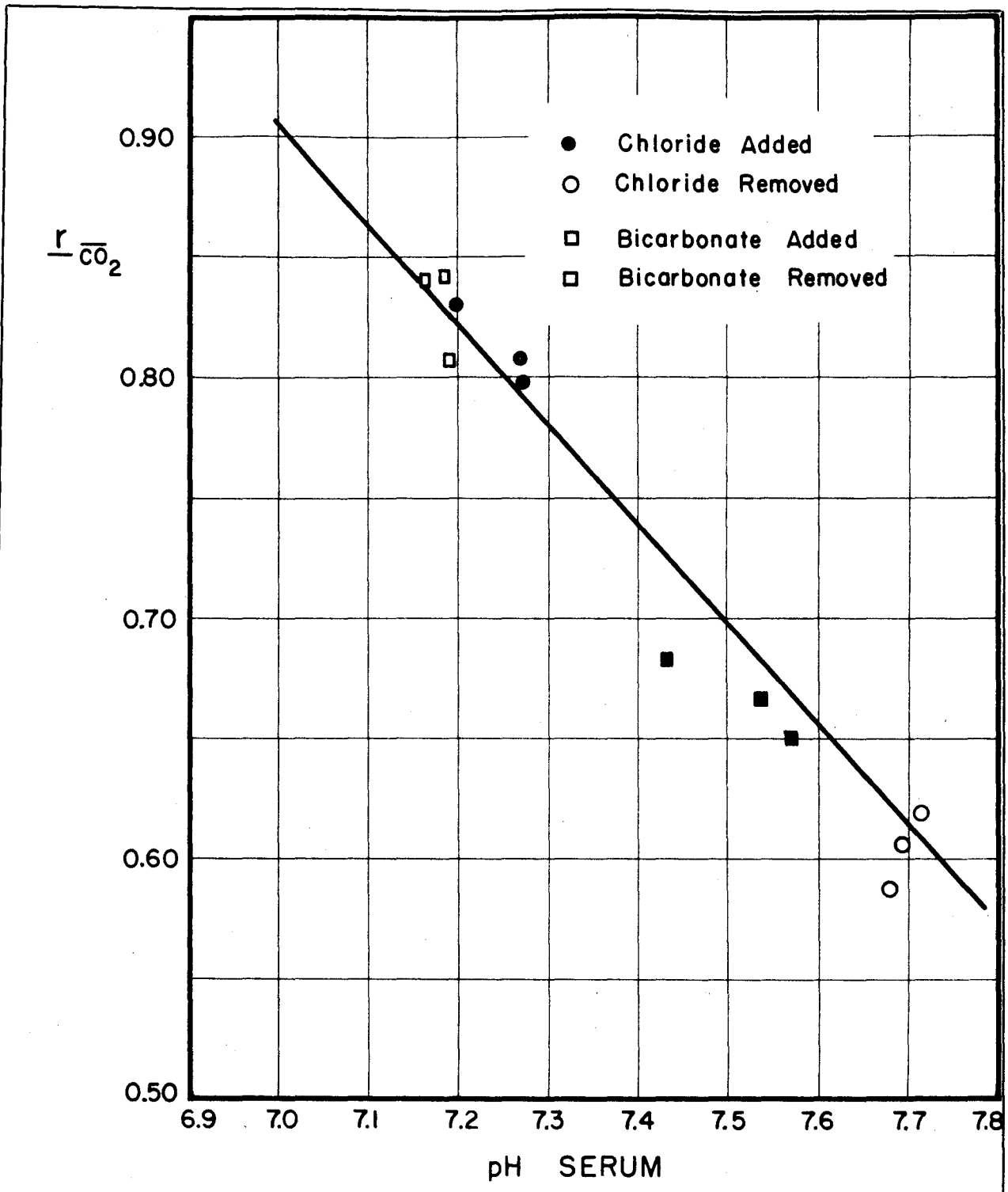

Fig. 11. The effect of alteration of the chloride or bicarbonate concentration upon the distribution ratio for $[\overline{CO}_2]$. The solid line represents the observed values for normal oxygenated blood.

manner in which the serum pH is changed. The experiments in which chloride is increased or decreased show that the relationship of $\underline{r}_{Cl}$ to serum pH is not affected by either action. The relationship of $\underline{r}_{\alpha H^+}$, $\underline{r}_{HCO_3}$, or $\underline{r}_{CO_2}$ to serum pH is likewise not affected.

If, as Peters' group (34) suggests, there are active and inactive fractions for the diffusible ions, the inactive fraction must be present in amounts so small that a fifty per cent change of the active fraction does not result in a change in the proportion of the inactive fraction sufficient to cause an appreciable effect in the distribution ratios. Therefore, if carbamate is an inactive fraction of the total combined-CO_2, its concentration is too large to permit the suggestion of Peters' group to be valid.

The experiments with isotonic sucrose solution offer some interesting information. From Table VII, $\underline{r}_{\alpha H^+}$, $\underline{r}_{Cl}$, $\underline{r}_{HCO_3}$, and $\underline{r}_{CO_2}$, determined in the usual manner, are calculated to have an average value of 1.080, 1.149, 1.220, and 1.354, respectively, at an average serum pH of 7.083 for oxygenated blood. The following relationship therefore exists:

$$(46) \qquad \frac{[\alpha H^+]_s}{[\alpha H^+]_c} = 0.94 \frac{[Cl]_c}{[Cl]_s} = 0.89 \frac{[HCO_3]_c}{[HCO_3]_s} = 0.80 \frac{[\overline{CO}_2]_c}{[\overline{CO}_2]_s}$$

This indicates apparent compliance with Donnan's law. The redistribution of chloride and bicarbonate, to restore the values of $\underline{r}$ to those of normal blood, which Peters' group looked for, and could not find, would require a transfer of potassium ions through the red blood cell membrane. This would be necessary so that electrical neutrality be maintained, since both chloride and

bicarbonate would be passing through the membrane in the same direction. However, the fact that the values of r differ from those of normal blood is explained, at least qualitatively, by the realization that the concentration of sodium ion present in the serum is reduced by dilution with isotonic sucrose solution. Since sodium ion is restrained, in some manner, from diffusing through the red blood cell membrane, it must be exerting a Donnan effect. (This would also be true of the potassium ions in the cells). Since the paper of Barcroft's group (5), workers seem to have disregarded this fact, possibly because serum sodium ions and cell potassium normally approximately are equal to each other and, thus, cancel the effect of one another. A decrease in serum sodium ions should result in a decrease in the Donnan effect of this nondiffusible positive ion and, consequently, a decrease in the tendence for an excess concentration of diffusible negative ions of serum over that of these negative ions in the cells. This may be more easily visualized by considering the sodium ion separately from the other nondiffusible ions, and by considering its effect on the distribution of the diffusible pair of ions, hydrogen and chloride. Thus, at equilibrium there would be present the following distribution:

Na^+	
H^+	H^+
Cl^-	Cl^-
(serum)	(cell)

where the straight line represents the red blood cell membrane. For the same reasons discussed concerning $[Na^+]$ and $[Cl^-]$ in the introduction of this paper, it follows that $[Cl^-]$ serum is greater than $[Cl^-]$ cell, and $[H^+]$ serum is less than $[H^+]$ cells. A decrease in the concentration of the sodium without a decrease of the same magnitude of the diffusible ions, because the cell constituents are not diluted, would result in a decrease of the extent of the unequal distribution of the diffusible ions. As a result of this change in sodium effect, the overall distribution in whole blood will be changed, so that the values of $\frac{[\text{diffusible cations}]_s}{[\text{diffusible cations}]_c}$ and $\frac{[\text{diffusible anions}]_s}{[\text{diffusible anions}]_c}$ will be increased. This is in agreement with the experimental data presented in this paper. Dilution of the serum protein would tend to have the reverse effect. However, its concentration is so small in comparison to that of the sodium ion that its effect should be almost negligible. Because sodium ion concentration before and after dilution with the isotonic sucrose solution was not determined, a quantitative estimation of the expected change in the distribution ratio, $\underline{r}$, cannot be made at this time.

Peters' group (34) objected to the use by Van Slyke's group of $\underline{r}_{\alpha_{H^+}}$ as a base line for the comparison of activity coefficient ratios because of the use of saponin to hemolyze the red blood cells in pH determinations, a treatment which Peters' group say "profoundly alters the state of the

saponin solution used in the experimental work of this paper was tested, first on a phosphate buffer solution at the pH range of blood, and then directly on cell hemolysate. The test with the phosphate buffer solution showed that the saponin solution in itself had no effect upon the pH being measured. The test with the cell hemolysate was carried out by preparing a suspension of cells from one day old blood, hemolyzed by alternate freezing and thawing, and transferring it to two vessels over Hg, one of which contained saponin solution in amount equivalent to that used for hemolysis in the experimental work of this paper. The pH of the two portions were then immediately measured in a glass electrode. No effect in the hydrogen ion activity, due to the presence of saponin, was observed.

Summary

1. The distribution of the diffusible ions, hydrogen, chloride, bicarbonate, and total combined $-CO_2$ between serum and cells of human blood has been studied over the pH range 7.0 to 7.8, in oxygenated and reduced blood.

2. It has been found that glycolysis has no effect on the distribution of these ions.

3. The experimentally determined distribution ratios in reduced blood at $pH_s = 7.4$ have been found to be related as follows:

$$\frac{[\alpha H^+]_s}{[\alpha H^+]_c} = 0.93 \quad \frac{[Cl]_c}{[Cl]_s} = 0.94 \quad \frac{[HCO_3]_c}{[HCO_3]_s} = 0.80 \frac{[\overline{CO}_2]_c}{[\overline{CO}_2]_s}$$

$[\alpha H^+]$ represents hydrogen ion activity, electrometrically determined, and $[Cl]$, $[HCO_3]$, and $[\overline{CO}_2]$ represent molalities in terms of mols of chloride, bicarbonate, and total combined $-CO_2$ per kilo of water.

4. The changes in the distribution of the diffusible ions studied between serum and cells with change in serum pH and in degree of oxygenation of the hemoglobin approximate those predicted by Van Slyke, Wu, and McLean, from the changes in base-binding power of the cell and serum proteins caused by varying pH and degree of oxygenation.

5. The addition or removal of chloride or bicarbonate does not affect the change of distribution ratio with change of serum pH.

6. Dilution of the serum with an isotonic solution of a non-diffusible un-ionized substance changes the value of the distribution ratios in a manner expected.

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